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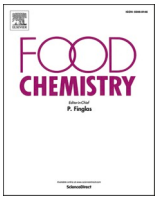

Review

Microbial and enzymatic strategies for aflatoxin control: Integrating intelligent detection and computational design

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ABSTRACT

Aflatoxins (AFs), potent carcinogenic mycotoxins, pose a major global threat to human health. This review offers an in-depth summary of microorganisms capable of degrading AFs, including bacteria, probiotics, and fungi, and highlights the key enzymes responsible for detoxification. We propose an integrated system combining smartphone-based detection, machine learning-driven enzyme discovery, and computationally optimized biocatalyst design for effective AFs mitigation. Microbial degraders facilitate aflatoxin B1 (AFB1) detoxification through extracellular enzymatic activity or cell surface adsorption mechanisms. Enzymes such as laccase, peroxidase, reductase, and lactonase effectively convert AFB1 into less toxic metabolites. However, industrial application of AFs-degrading enzymes remains constrained by their instability and insufficient efficiency. Emerging technologies, including machine learning-driven enzyme discovery and computer-aided protein engineering demonstrate significant potential for enhancing enzyme performance. This review highlights that integrating intelligent detection systems with computer-aided enzyme design offers a transformative framework for proactive AF control throughout food and feed supply chains.

1. Introduction

Aflatoxins (AFs) are secondary metabolites synthesized by filamentous fungi, primarily *Aspergillus flavus* and *A. parasiticus*, representing a significant global public health and economic threat (Cao et al., 2022). Several AFs have been identified, including aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), and aflatoxin M1 (AFM1), with AFB1 being the most carcinogenic and classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC) (Jallow et al., 2021). These mycotoxins are synthesized throughout the entire cereal production chain-from preharvest through processing stages-subsequently entering food and feed chains and posing substantial risks to human and animal health (Hao et al., 2023a).

The biosynthesis of AFs is conducted by at least 27 enzymes and regulated by transcription factors *aflR* and *aflS* (Caceres et al., 2020). The first stable precursor in the AFs biosynthetic pathway is norsolorinic acid (NOR), which is synthesized from acetate units by a non-reducing polyketide synthase (Yu, 2012). The identification of NOR enabled the isolation of the first AFs pathway gene, which ultimately lead to AFs production. In *A. flavus* and *A. parasiticus*, the AFs biosynthetic genes are organized in a cluster within a 75-kb region on chromosome III, located approximately 80 kb from the telomere (Yu et al., 2004). The AFs outbreaks occurs in a wide range of crops, such as maize, wheat, cereal, and peanuts. Commodities derived from AFs-contaminated crops present serious public health concerns due to their established mutagenic, tumorigenic, and carcinogenic properties (Benkerroum, 2020). Furthermore, when livestock consume feed contaminated with AFs, residues of AFs can persist in animal-derived products, especially milk and dairy products, which further endanger human health (Guo et al., 2019).

Numerous physical, chemical, and biological technologies have been developed to mitigate AF contamination in crops, food, and feed (Chu et al., 2017; Luo et al., 2014). For example, montmorillonite is commonly used in animal feed as anti-caking agents for mycotoxin adsorption (Vila-Donat et al., 2018). In addition, biological methods, especially enzymatic degradation, are gaining attention for their specificity, environmentally friendly byproducts, and potential for integration into food/feed processing systems. In this review, we aim to present a comprehensive and critical analysis of microbial and enzymatic strategies for AFs degradation. We systematically trace the progression from

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microbial screening and AFs-degrading enzyme identification to molecular engineering, emphasizing the role of computational approaches in enhancing enzymatic efficiency and stability. Recent studies in the mycotoxin field have highlighted advances in smartphone-based rapid detection platforms, comprehensive enzyme-mycotoxin interaction databases, and deep learning-based discovery of mycotoxin-degrading enzymes. In the future, integrating these technologies will be critical for AFs management, enabling intelligent, real-time monitoring and targeted deployment of biocatalysts to achieve faster, more precise, and sustainable AFs remediation.

2. Biosynthesis, occurrence and metabolism of AFs

2.1. Biosynthesis of AFs

AFs biosynthesis in *Aspergillus* species proceeds via a complex polyketide pathway comprising at least 27 enzymatic steps. The complex polyketide pathway converts acetate and malonate building blocks into complex furanocoumarin structures through a series of oxidation, reduction, cyclization, and methylation steps. The genes encoding these enzymes are organized in a 54th cluster on chromosome 3 in *Aspergilli* and are regulated by the pathway-specific transcription factors *aflR* and *aflS* (Caceres et al., 2020). The pathway initiates with the condensation of acetate units catalyzed by a non-reducing polyketide synthase (PKS) encoded by *aflC* (also known as *pksA*), forming a norsolorinic acid (NOR) backbone (Fig. 1). NOR is then converted through sequential reactions into averantin (AVN), versicolorin A (VERA), sterigmatocystin (ST), and ultimately AFB1 and AFG1 through the activity of monooxygenases, dehydrogenases, methyltransferases, and oxidoreductases (Yu, 2012).

Importantly, environmental and physiological conditions play a major role in modulating gene expression within the cluster. pH, carbon and nitrogen sources, temperature, oxidative stress, and even light exposure have all been reported to affect the expression of *afl* genes and, consequently, AFs production (Yu, 2012). For example, oxidative stress induced by reactive oxygen species (ROS) has been shown to upregulate *aflR* and AFs synthesis, possibly linking AFs production to fungal stress responses (Reverberi et al., 2006). Moreover, the optimum temperature for AFs production is in the range from 28 to 35 °C (Obrian et al., 2007). High temperature inhibits the transcription factor *aflR* for transcription activation. AFs affection outbreaks in crops occurred under hot weather and drought conditions (Cotty et al., 2007). Therefore, climate conditions significantly influence AFs contamination, with moisture and warm environments favoring their production (Fig. 2A) (Jallow et al., 2021). Tropical and subtropical crops are particularly vulnerable to *Aspergillus* and AFs contamination.

2.2. Occurrence and consumption of AFs

As a populous nation encompassing vast tropical and subtropical regions, China faces prolonged and widespread exposure of its people to AFs (Woo & El-Nezami, 2015). The Chinese government has implemented regulatory standards such as GB 2761–2017 (China Food and Drug Administration, 2017) to limit AFs presence in crops and foodstuffs, as outlined in Table 1. Nevertheless, numerous studies have documented persistent AFs contamination in various crops. For instance, maize samples from North China in 2022 contained average total AFs concentrations of 22.0 μg/kg (Cheng et al., 2022), whilst a Shanghai survey (2008–2011) identified AFB1 in 0.81 % of cereal-based products, with concentrations ranging from 0.5 to 47.3 μg/kg (Yang et al., 2020). Peanut samples exhibited a 13.24 % detection rate for total AFs, with concentrations reaching up to 356.7 μg/kg (Qin et al., 2021a). Moreover, the wheat samples from Spain exhibited a 23 % detection rate for AFB1, with concentrations ranging from 1.03 to 9.50 μg/kg (Jallow et al., 2021).

AFB1 contamination inevitably permeates the food chain, with concentrations in market rice samples ranging from 1.45 to 17.71 μg/kg, occasionally exceeding the 10.0 μg/kg regulatory limit (Sun et al., 2017). In Nigeria, AFs were detected in 100 % of 100 roasted cashew nut samples, with concentrations ranging from 0.1 to 0.68 μg/kg. (Jallow et al., 2021). Similarly, 48.3 % of soybean-related products contained AFB1 (0.36–11.26 μg/kg) (Zhang et al., 2024a). AFs contamination appears more pronounced in animal feed, with total AFs detected in 26.4 % of dried distillers' grains samples (mean concentration: 31.94 μg/kg) (Hao et al., 2023a). Animals consuming contaminated feed subsequently produce AFs-contaminated products, perpetuating the contamination cycle. For example, AFM1 was detected in 62.5 % of raw buffalo milk samples (4–243 ng/kg) and 74.4 % of dairy products (4–235 ng/kg) (Guo et al., 2019). This cyclical contamination pattern throughout the food chain ultimately threatens human health, as illustrated in Fig. 1B.

2.3. Metabolism of AFs in liver

Following ingestion by animals, AFs are absorbed and transported to their primary target organ, the liver, where AFB1 undergoes bioactivation by cytochrome P450 (CYP450) enzymes, as illustrated in

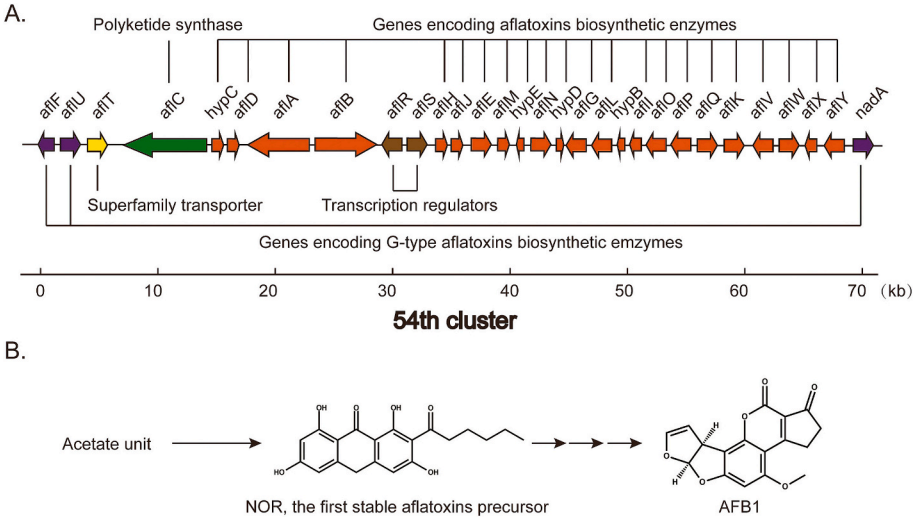

Fig. 1. Biosynthetic pathway of AFB1. (A) The aflatoxin biosynthetic gene cluster (Cluster 54) located on chromosome 3 in *Aspergillus* species. (B) The biosynthetic pathway from acetate to the first stable aflatoxin precursor (NOR), and ultimately to AFB1.

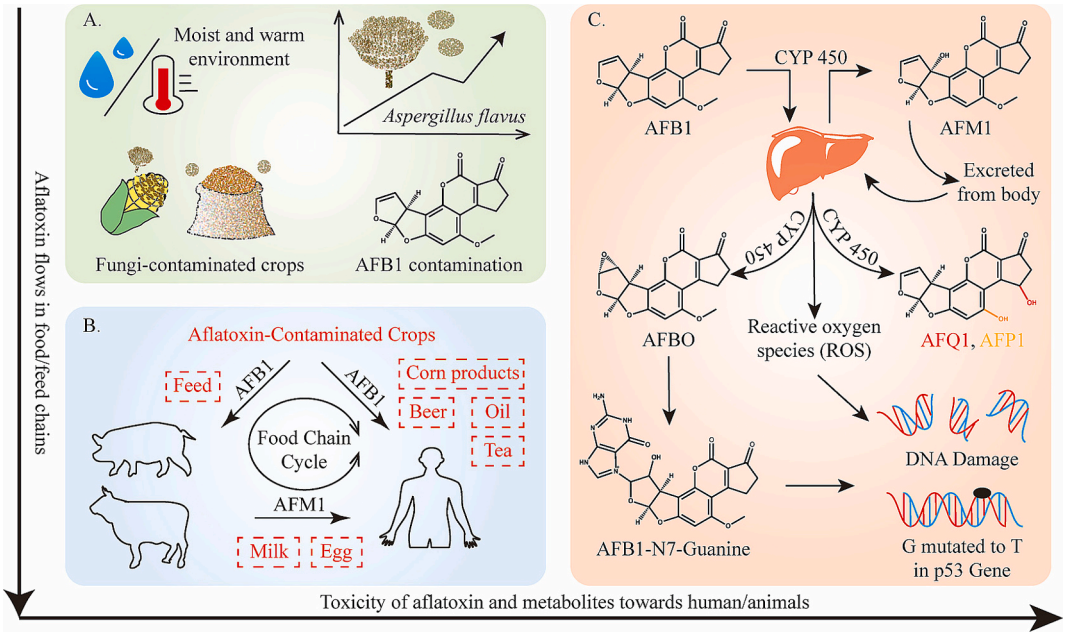

Fig. 2. Generation, contamination, and toxicity of AFB1. (A) The production of AFB1 by *Aspergillus* genus under moist and warm environment. (B) AFB1 contamination in food and feed supply chain, and main AFB1-contamianted products. (C) metabolic mechanisms and toxicity of AFB1 in its primary organ liver. Abbreviation of metabolites: AFM1: aflatoxin M1; AFBO: AFB1–8,9-*exo*-epoxide; AFQ1: aflatoxin Q1; AFP1: aflatoxin P1; AFB1-N7-guanine: N7-guanine adduct trans-8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1.

Table 1
Recent aflatoxin exposure assessments in China.

Matrix	Detection of aflatoxin	Incidence (sample size)	Detection of aflatoxin concentration	Limitation of aflatoxin B1 (μg/kg) in GB 2761–2017	Reference
Maize	Total AF	8.0 % (426)	22.0 μg/kg in maize	20	(Cheng et al., 2022)
Rice	AFB1	5.6 % (161)	1.45–17.71 μg/kg in rice from markets	10	(Sun et al., 2017)
Wheat, and barley	Total AF	1.2 % (411)	2.6 μg/kg in wheat	5.0	(Hao et al., 2023)
Wheat flour, and cereal	AFB1	0.81 % (1980)	0.5–47.3 μg/kg in cereals and cereal-based products in Shanghai from 2008 to 2011	5.0	(Yang et al., 2020)
Beans and bean products	AFB1	48.3 % (203)	0.36–11.26 μg/kg in soybean-related products	5.0	(Zhang et al., 2024a)
Peanuts and their products	Total AF	13.24 % (929)	< 356.7 μg/kg in peanuts	20	(Qin et al., 2021a)
Peanut oil, and corn oil	AFB1, and total AF	66.6 % (30)	36.2 μg/kg, and 44.4 μg/kg in peanut oil	20	(Li et al., 2023)
Spices	AFB1	75 % (43)	26.2 μg/kg in red chilli powder from markets	[α]NR	(Bi et al., 2023)
Condiment (soy sauce, vinegar, brewing sauce)	AFB1	99.4 % (929)	0–16.41 μg/kg in Doubanjiang (a famous Chinese condiment)	5.0	(Zhang et al., 2020)
Milk and milk products	AFM1	74.4 % (86) 62.5 % (136) 82.8 % (516) 59 % (329)	4–235 ng/kg in dairy products 4–243 ng/kg in raw buffalo milk 5.1–85.2 ng/L in milk 10.0–66.7 ng/L in yogurt	0.5	(Guo et al., 2019) (Xiong et al., 2022)
Tea	AFB1	1.27 % (158)	In Chinese post-fermented dark tea	NR	(Cui et al., 2020)
Feed	AFB1	16.0 % (1610)	34 μg/kg in feeds and raw materials (maximum at 482 μg/kg)	NR	(Li et al., 2022)
	Total AF	82.6 % (9392) 26.4 % (197) 29.99 % (1857) 21.93 % (1418)	103.08 μg/kg in new season corn 31.94 μg/kg in DDGS 15.79 μg/kg in poultry feed 16.95 % in swine feed	NR	(Hao et al., 2023)

[α] NR: Not reported.

Fig. 2C. CYP450 enzymes, particularly CYP1A2 and CYP3A4, oxidize the C=C double bond in the furan ring of AFB1, generating more toxic metabolites (Loi et al., 2020). This oxidation process involves the insertion of an oxygen atom into the double bond, creating an unstable epoxide, notably AFB1–8,9-*exo*-epoxide (AFBO). AFBO subsequently alkylates DNA bases and covalently binds to nucleophilic sites in DNA, forming a stereospecific N7-guanine adduct trans-8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1 (AFB1-N7-gua) (Qin et al., 2021a). Significantly, the formation of AFB1-N7-gua adducts can induce guanine to thymine (G → T) transversions at the third nucleotide of codon 249 in the tumor suppressor *p53* gene, a mutational hotspot strongly associated with hepatocellular carcinoma (HCC) in high-exposure populations.

Furthermore, approximately 20 % of unstable AFB1-N7-gua adducts undergo depurination or rearrangement to form persistent AFB1-formamidopyrimidine adducts (AFB1-FAPy) (Cao et al., 2022), which demonstrate persistent mutagenic potential in vivo. Studies conducted in *Escherichia coli* have revealed that AFB1-FAPy adducts induce G → T transversion frequencies approximately six times greater than those induced by AFB1-N7-gua adducts (Cao et al., 2022).

In the liver, AFB1 can also be metabolized into AFM1 by CYP450 enzymes, which is subsequently excreted via urine and faeces. AFM1 may be secreted into milk and eggs, contributing to its detection in dairy products and food chains (Guo et al., 2019). Although AFM1 exhibits significantly lower genotoxicity than AFB1, the IARC categorizes it as a Group 2B human carcinogen based on evidence of carcinogenicity in animal models. Additionally, CYP3A4 can catalyze the conversion of AFB1 to AFQ1 through epoxidation and hydroxylation reactions, whilst CYP2A13, CYP2A3 and CYP321A1 facilitate the formation of AFP1 via demethylation (Cao et al., 2022). AFQ1 demonstrates excretion levels 60 times higher than AFM1 in faeces and urine. Moreover, AFP1 lacks mutagenic potential compared to parent AFB1, as evidenced by fertile egg toxicological studies (Cao et al., 2022). Beyond its metabolites, AFB1 also promotes the generation of reactive oxygen species (ROS), including peroxynitrite, hydrogen peroxide, superoxide, and hydroxyl radicals (Shi et al., 2024). Excessive ROS production can induce oxidative stress through DNA lesions and disrupt mitochondrial function via ROS-dependent permeability transition.

3. Degradation of AFs by microorganisms

3.1. Isolation of AFs-degrading microorganisms

Microbial degradation of AFs represents a promising bioremediation strategy. Over the past few decades, numerous microbial strains capable of degrading AFs have been isolated from various environmental sources, including soil (Xia et al., 2017), AFs-contaminated tea leaves (Fang et al., 2020), animal faeces (Ali et al., 2021), contaminated crops, fermented foods (Petchkongkaew et al., 2008), and decaying bark (Ning et al., 2019). Screening methodologies for isolating AFs-degrading microorganisms from environmental samples typically utilize coumarin as the sole carbon source to selectively enrich AFs-metabolizing strains. Subsequent steps may include iterative enrichment, 16S rRNA-based phylogenetic analysis, confirmation of functional genes or metabolites, and product identification, as shown in Fig. 2. As AFs belong to a class of bisfuranocoumarin derivatives, they share a conserved coumarin core within their molecular structure (Guan et al., 2008). Consequently, microorganisms capable of metabolizing coumarin may also exhibit the ability to degrade AFs (Fig. 3). Numerous AFs-degrading microorganisms have been isolated using coumarin as the sole carbon source (Guan et al., 2008; Guo et al., 2024; Shu et al., 2018).

3.2. Degradation of AFs by bacteria

Among the AFs-degrading bacteria, *Rhodococcus erythropolis*, enriched from agricultural soil, exhibits significant AFB1 degradation

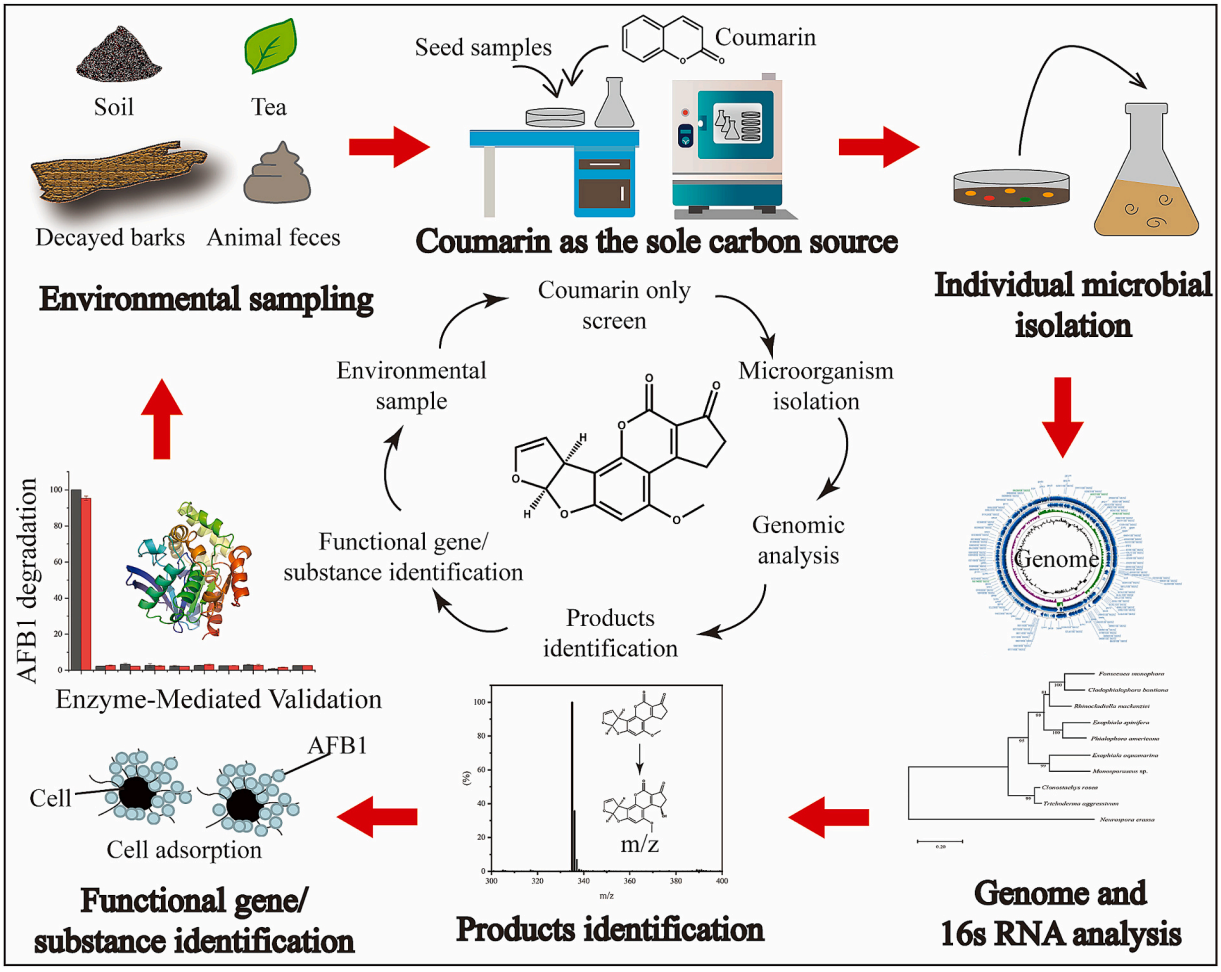

Fig. 3. Screening, characterization, products identification, and functional gene or substance identification of AFs removal microorganisms by utilize coumarin as the sole carbon source.

efficiency (>90 % within 72 h) (Risa et al., 2018). Moreover, *Pseudomonas anguilliseptica* VGF1, isolated from gold mine aquifer, demonstrated significant biodegradation capability by reducing 66.5 % of 0.5 mg/L AFB1 within 2 days (Adebo et al., 2016a). Cytotoxicity studies revealed that bacteria-treated products exhibited substantially lower toxicity to human lymphocytes compared to untreated AFB1, indicating that *P. anguilliseptica* VGF1 effectively disrupts the toxic molecular structure of AFB1. Similarly, *P. putida* MTCC2445 showed remarkable efficiency by degrading 90 % of 0.2 mg/L AFB1 within 24 h (Samuel et al., 2014). Analysis of degradation products revealed that *P. putida* MTCC2445 transforms AFB1 into AFD1, AFD2, and AFD3, all exhibiting reduced toxicity against HeLa cells. Further investigations suggested that extracellular enzymes from *P. putida* MTCC2445 likely facilitate lactone ring opening in AFB1. Additionally, *Stenotrophomonas* sp. CW117 achieved complete degradation of 4 mg/L AFB1 within 48 h, yielding products with no detectable biotoxicity to *Vibrio fischeri* DH22 (Cai et al., 2020). The recently isolated *P. aeruginosa* M-4 from rotten wood has shown considerable potential, with its culture supernatant achieving 56.79 % AFB1 degradation, producing compounds identified as $C_{17}H_{16}O_6$, $C_{16}H_{14}O_5$, $C_{17}H_{14}O_5$, and $C_{16}H_{10}O_6$ (Xu et al., 2023). Within the *Bacillus* genus, several species have demonstrated notable efficacy in degrading AFs. *B. amyloliquefaciens* achieved an 80.9 % degradation rate of AFB1 within 3 days, converting it to less toxic AFD1 (Shi et al., 2024). *B. subtilis* RWGB1 and *B. oceanisediminis* RWGB2 attained degradation rates of 84.2 % and 66.5 %, respectively, within 2 days (Shi et al., 2024). Particularly noteworthy, *B. velezensis* DY3108 exhibited exceptional performance by degrading 91 % of AFB1 in liquid media, likely due to extracellular enzymatic activity (Shu et al., 2018). Further characterization of degradation metabolites and their cytotoxicity remains essential for validating detoxification safety and industrial applicability.

3.3. Degradation of AFs by fungus

Fungal species also play a crucial role in AFs degradation through various mechanisms. As summarized in Table 2, *A. niger* FS10, isolated from fermented Chinese soybean, demonstrated remarkable efficiency by degrading 98.6 % of AFB1 within 3 days via glutathione-mediated biotransformation (Qiu et al., 2021). The resulting product, formed through lactone ring cleavage of AFB1, likely exhibits reduced toxicity. Similarly, *Pleurotus ostreatus* GHBBF10, obtained from decomposing tree trunk, converted AFB1 to less toxic AFB2a after 15 days of incubation (Das et al., 2014). In contrast to microbial biotransformation, direct binding of AFs to fungal cell surfaces offers a more rapid approach to AFB1 removal. For instance, *Saccharomyces cerevisiae* can remove AFB1 through cell wall β-glucan adsorption, achieving 65 % removal efficiency within just 3 h (Shetty et al., 2007). This rapid binding mechanism relies on interactions between fungal polysaccharides and AFB1, particularly through the single helix structure of (1 → 3)-β-D-glucan chains and branched (1 → 6)-β-D-glucan chains in the yeast cell wall. Modification of cell wall surface components can significantly enhance binding efficiency; heat treatment and esterification of oligomannans on *S. cerevisiae* cell surfaces increased AFB1 removal efficiency from 40 % to 95 % (Shetty et al., 2007). Another yeast, *Komagataella pastoris* EW1, removes AFB1 via cellular adsorption, achieving 71.5 % adsorption within 5 days (García-Béjar et al., 2020). Edible fungi have also been explored for AFs detoxification, leveraging their enzymatic capabilities. *Armillariella tabescens* GC-Ac2 has demonstrated efficacy in converting AFB1 into less toxic metabolites (C. Guo et al., 2024). The culture supernatants of *A. tabescens* GC-Ac2 exhibited manganese peroxidase (MnP) activity, suggesting enzymatic involvement in AFB1 degradation.

3.4. Degradation of AFs by probiotics

Beyond conventional fungi and bacteria, probiotics have emerged as safe, multifunctional agents for AFs removal, combining detoxification capabilities with host health benefits. *Bifidobacterium breve* Bbi99/E8, for example, can adsorb 23 % of 4 mg/L AFB1 within just 1 h whilst simultaneously providing health benefits to the host (Halttunen et al., 2008). The removal mechanism involves hydrophobic interactions between the bacterial cell surface and AFB1, facilitated by carbohydrates and proteins that enable efficient capture of the toxin. Similarly, *Bifidobacterium angulatum* isolated from kefir grains achieved 23.6 % AFB1 removal within 5 h through physical adsorption by its cell wall (Elsanhoty et al., 2016). Notably, the haloduric lactobacillus *Tetragenococcus halophilus* CGMCC 3792 employs a different strategy, converting AFB1 into six non-toxic metabolites through distinct intracellular components rather than cell wall adsorption (Li et al., 2018a). Further investigations involving proteinase K and SDS treatment indicated that intracellular enzymes in *T. halophilus* CGMCC 3792 likely contribute to AFB1 degradation. Additionally, *Enterococcus faecium* HB2–2, a potential probiotic, demonstrated impressive performance by degrading 82.9 % of AFB1 in contaminated peanut meal over 96 h under conditions of pH 10 and 32 °C, reducing residual AFB1 levels from 105.1 to 17.9 μg/kg (Feng et al., 2024).

3.5. Degradation of AFs by hybrid microorganisms

The combined application of multiple microorganisms for AFs removal offers enhanced effectiveness through complementary biodegradation and biosorption mechanisms. Kombucha culture, comprising multiple strains including *Pichia occidentalis*, *Candida sorboxylosa*, and *Hanseniaspora opuntiae*, demonstrates this synergistic approach by both adsorbing AFB1 and transforming it into metabolic derivatives (Ben Taheur et al., 2020). Within this microbial consortium, yeasts play a more significant role in AFB1 adsorption than bacteria. Cytotoxicity studies on Hep2 cells and brine shrimp demonstrated substantially reduced toxicity of metabolites from Kombucha-treated AFB1, highlighting the promise of multi-organism approaches for mitigating AFs contamination in food and feed matrices. The functional components responsible for AFs removal primarily consist of intracellular substances (particularly enzymes) or cell wall components (carbohydrates and proteins). Whilst intracellular enzymes convert AFs into less toxic derivatives through biotransformation, cell surface components facilitate AFs removal through adsorption mechanisms.

4. Enzymatic degradation for AFs

4.1. Enzymatic products and mechanisms

Enzymes serve as pivotal agents in the biotransformation of AFB1 by microorganisms, offering a safer alternative to whole microbial applications by eliminating the risks associated with potentially harmful strains. The products and mechanisms of the reported enzymes have been identified and hypothesized (Fig. 4). Many degradation products have been confirmed to be less toxic than their parent compounds. For instance, laccase from *Trametes* sp. C30, when recombinantly expressed in *S. cerevisiae*, achieves 91 % AFB1 degradation within 15 h (Liu et al., 2021). Another laccase derived from *Weizmannia coagulans* 36D1, Lac—W, exhibits broad-spectrum mycotoxin-degrading activity, efficiently degrading 88 % AFB1, 60 % zearalenone (ZEN), and 34 % deoxynivalenol (Jia et al., 2024). The degradation product of AFB1 treated with Lac-W was identified as AFQ1 (Hao et al., 2023b). Moreover, *Ba*DyP and *Bs*DyP oxidize the double bond on the furan ring of AFB1, converting it into AFB1-diol by adding two hydroxyl groups to the AFB structure (Qin et al., 2021b; Shao et al., 2024). This AFB1-diol exhibits significantly reduced cytotoxicity compared to AFB1, as demonstrated through in vitro assays using HepG2 cells (Shao et al., 2024). Cell viability of HepG2 cells exposed to *Bs*DyP-treated AFB1 increased substantially from 33 % to 60 % compared to untreated AFB1. Additionally, several enzymes including ADPP III (Zhang et al., 2024b), AttM (Cheng et al., 2023), BacC (Afsharmanesh et al., 2018), and peroxiredoxin

Table 2
Comprehensive summary of detoxifying ability of AFB1-removing microorganisms and their sources.

Microorganisms	Sources of matrices	Removal rate	Degradation substance or mechanism	Products and toxicity	Reference
Bacteria					
Bacillus amyloliquefaciens	Kimchi	80.9 % AFB1 in 3 d	Enzymes	The product AFD1 exhibited less cytotoxic than AFB1	(Shi et al., 2024)
Bacillus halotoleran DDC-4	Moldy maize and rice	76.3 % AFB1 (1 mg/L) in 3 d	Enzymes	NR	(Guo et al., 2024)
Pseudomonas aeruginosa M-4	Rotten wood	56.8 % AFB1 (2.5 ng/mL) in 14 d	Enzymes	The products showed lower toxicity than AFB1	(Xu et al., 2023)
Pseudomonas anguilliseptica VGF1	Gold mine aquifer	66.5 % AFB1 (0.5 mg/L) in 2 d	Enzymes	The cytotoxicity studies against human lymphocytes showed less toxicity of products	(Adebo, Njobeh, Sidu, et al., 2016)
Pseudomonas fluorescens		63.0 % AFB1 (0.5 mg/L) in 2 d			
Staphylococcus sp. VGF2		100 % AFB1 (0.5 mg/L) in 2 d			
Bacillus velezensis DY3108	Soil samples	94.7 % AFB1 (0.5 mg/L) in 4 d	Culture Supernatant (protein or enzyme)	The cytotoxicity assays showed lower cytotoxicity of products	(Shu et al., 2018)
Pseudomonas putida MTCC1072	Obtained from MTCC Chandigarh, India	80 % AFB1 (0.2 mg/L) in 24 h	Enzyme	Products AFD1 and AFD2 are much less toxic than AFB1 due to the opening of lactone ring	(Singh & Mehta, 2019)
Burkholderia sp.	Corn soil samples	85 % AFB1 (2.5 mg/L) in 60 h	Extracellular enzyme	[a]NR	(Singh & Mehta, 2019)
Stenotrophomonas sp. CW117	PAH-polluted soil near a refinery	100 % AFB1 (4 mg/L) in 2 d	Culture supernatant	The degradation product showed no biotoxicity to *Vibrio fischeri* DH22	(Cai et al., 2020)
Bacillus subtilis RWGB1	Rice weevils	84.2 % AFB1 (1 mg/L) in 2 d	NR	NR	(Al-Saadi et al., 2024)
Bacillus oceanisediminis RWGB2		66.5 % AFB1 (1 mg/L) in 2 d			
Pseudomonas aeruginosa		48.9 % AFB1 (1 mg/L) in 2 d			
Rhodococcus strains	Japan Collection of Microorganism	90 % AFB1 (3 mg/L) in 3 d	NR	The products showed lower genotoxicity than AFB1	(Risa et al., 2018)
Rhodococcus rhodochorus NI2	Hydrocarbon-contaminated sites	>90 % AFB1 (4 mg/L) in 3 d	Metabolic activities of microorganism	The product has no cytotoxic effect on *Aliivibrio fischeri*	(Krifaton et al., 2011)
Rhodococcus erythropolis ATCC4277	Obtained from the Institute of Pharmacy and Biomedical Sciences (SIPBS)	95.9 % AFB1 (20 mg/L) in 24 h	Metabolic activities of microorganism	NR	(Eshelli et al., 2015)
Streptomyces lividans TK24		87.9 % AFB1 (20 mg/L) in 24 h			
Streptomyces aureofaciens ATCC10762		86.1 % AFB1 (20 mg/L) in 24 h			
Streptomyces cacaoi subsp. *asoensis* K234	Soil, decaying plant parts, peat moss and compost samples	88.3 % AFB1 (1 mg/L) in 5 d	NR	Genotoxicity of products remained high	(Harkai et al., 2016)
Bacillus subtilis UTBSP1	Pistachio nuts	78.3 % AFB1 (2.5 mg/L) in 3 d	Cell free supernatant	NR	(Farzaneh et al., 2012)
Bacillus subtilis BCC42005	Fermented cereal products	40 % AFB1 (0.2 mg/L) in 7 d	Extracellular fraction	NR	(Watanakij et al., 2020)
Bacillus subtilis JSW-1	Soil	62.8 % AFB1 (2.5 mg/L) in 3 d	Extracellular proteins or enzymes	NR	(Xia et al., 2017)
Escherichia coli 12–5	Soil samples from pesticide company	58.8 % AFB1 (0.1 mg/L) in 4 d	Culture supernatant	NR	(Elaasser & El Kassas, 2011)
Pseudomonas putida 12–3		69.3 % AFB1 (0.1 mg/L) in 4 d			
Microbial consortium, TADC7 (*Geobacillus* and *Tepidimicrobium* genus)	Agricultural waste	31 % AFB1 (5 mg/L) in 3 d	Enzymes	NR	(Wang et al., 2017)
Bacillus albus YUN5	Traditional Korean food (doenjang)	76.3 % AFB1 (2 mg/L), 98.9 % AFG1 (2 mg/L) in 7 d	Enzymes	NR	(Kumar et al., 2023)
Bacillus licheniformis	Thai fermented soybean (Thua-nao)	74 % AFB1 (5 mg/L) in 5 d	NR	NR	(Petchkongkaew et al., 2008)
Bacillus subtilis		85 % AFB1 (5 mg/L) in 5 d			
Bacillus licheniformis CFR1	Agricultural soils	94.7 % AFB1 (0.5 mg/L) in 3 d	Culture supernatant	The products lost the mutagenicity of AFB1 based on Ames test	(Raksha Rao et al., 2017)
Bacillus shackletonii L7	Soil	92.1 % AFB1 (0.5 mg/L), 84.1 % AFBB2, 90.4 % AFM1 in 3 d	Enzyme	The products showed lower genotoxicity than AFB1	(Xu et al., 2017)
Bacillus mojavensis RC3B	Pond mud and soil samples	55.5 % AFB1 (0.2 mg/L) in 3 d	Enzyme	The toxicity of product to *Artemia salina* was lower than that of AFB1	(González Pereyra et al., 2019)

(*continued on next page*)

Table 2 (*continued*)

Microorganisms	Sources of matrices	Removal rate	Degradation substance or mechanism	Products and toxicity	Reference
Bacillus sp. TUBF1	Grains of corn plant	100 % AFB1 (10 mg/L) in 3 d	Crude enzyme	Bioassay against *Artemia salina* showed lower toxicity of products than that of AFB1	(El-Deeb et al., 2013)
Bacillus pumilus E-1-1-1	African elephants	89.5 % AFM1 (0.4 mg/L) in 12 h	Culture supernatant	NR	(Gu et al., 2019)
Lysinibacillus fusiformis	Gold mine aquifer	61.3 % AFB1 (2.5 mg/L) in 2 d	Intracellular protein	The cytotoxicity study against human lymphocytes showed lower toxicity of products	(Adebo, Njobeh, & Mavumengwana, 2016)
Staphylococcus warneri		47.7 % AFB1 (2.5 mg/L) in 2 d			
Sporosarcina sp.		46.9 % AFB1 (2.5 mg/L) in 2 d			
Escherichia coli CG1061	Chicken cecum	93.7 % AFB1 (2.5 mg/L) in 3 d	Intracellular protein	The products showed lower toxicity than AFB1 according to in vitro experiments on chicken hepatocellular carcinoma (LMH) cells and in vivo experiments on mice,	(Wang et al., 2019a)
Pseudomonas putida MTCC 1274 *Pseudomonas putida* MTCC 2445	Obtained from MTCC Chandigarh, India	90 % AFB1 (0.2 mg/L) in 24 h	NR	Products AFD1, AFD2, and AFD3, showed lower toxicity toward HeLa cells	(Samuel et al., 2014)
Yeast					
Agrocybe cylindracea	Purchased from the CGMCC	95.4 % AFB1 (0.5 mg/L) in 37.9 h	Enzyme	AFB1 was degraded into non-toxic products	(C. Guo et al., 2024)
Candida versatilis CGMCC 3790	Soy sauce mash	69.4 % AFB1 (20 mg/L) in 1 h	Biodegradation	The structure of products showed less toxicity than AFB1	(Li et al., 2018b)
Kluyveromyces lactis CBS 2359 + *Saccharomyces cerevisiae* ATCC 9763	Provided by others	95.5 % AFB2 (20 mg/L) in 3 d	NR	The cytotoxicity studies against human fibroblasts showed 10 times lower cytotoxicity of products	(Moustafa et al., 2017)
Komagataella pastoris EW1	Unpublished data	71.5 % AFB1 (40 μg/L) in 5 d	Cellular adsorption	NR	(García-Béjar et al., 2020)
Saccharomyces cerevisiae	Fermented maize dough and sorghum beer	>65 % AFB1 (1 mg/L) in 3 h	Binding AFB1 by microorganism	NR	(Shetty et al., 2007)
Aspergillus niger FS10	Fermented chinese soybean	98.6 % AFB1 (1 mg/L) in 3 d	Glutathione-mediated biotransformation	The structure of products indicated the lower toxicity of products than that of AFB1	(Qiu et al., 2021)
Pichia norvegensis	Tofu wastewater	36.9 % AFB1 (5.4 mg/L) and 27.1 % AFB2 (0.17 mg/L)	β-glucan-mediated biotransformation	NR	(Utama et al., 2021)
Pleurotus eryngii ITEM13681	Obtained from the Institute of Sciences of Food Production	90 % AFB1 (0.5 mg/L) in 10 d	NR	NR	(Branà et al., 2017)
Pleurotus ostreatus GHBBF10	Decomposing tree trunk	91.7 % AFB1 (0.5 mg/L) in 15 d	Enzymes	AFB1 was converted to less toxic AFB2a	(Das et al., 2014)
Pleurotus ostreatus MTCC 142		89.1 % AFB1 (0.5 mg/L) in 15 d			
Pleurotus ostreatus N001	Obtained from the Spanish Type Culture Collection	94 % AFB1 (2.5 mg/kg) in 6 weeks	NR	The mutagenicity of products was minimal based on the Ames mutagenicity assay	(Jackson & Pryor, 2017)
Probiotic					
Enterococcus faecium HB2–2	Grassland soil	82.9 % AFB1 (105.1 μg/kg in peanuts meal) in 4 d	Enzyme	The cytotoxicity of products was significantly lower than that of AFB1	(Feng et al., 2024)
Bifidobacterium angulatum	Purchased from institutes	23.6 % AFB1 (2 g/L) in 5 h	Physical adsorption of bacterial surface	NR	(Elsanhoty et al., 2016)
Lactobacillus plantarum		19.9 AFB1 (2 g/L) in 5 h			
Lactobacillus acidophilus		18.6 AFB1 (2 g/L) in 5 h			
Lactobacillus rhamnosus		19.56 % AFB1 (2 g/L) in 5 h			
Streptococcus thermophiles		14.9 % AFB1 (2 g/L) in 5 h			
Bifidobacterium breve Bbi99/E8	Purchased from institutes	23 % AFB1 (4 mg/L) in 1 h	Hydrophobic interactions between bacteria and AFB1	NR	(Halttunen et al., 2008)
Lactobacillus rhamnosus GG		~10 % AFB1 (4 mg/L) in 1 h			
Propionibacterium freudenreichii shermanii JS		13 % AFB1 (4 mg/L) in 1 h			
Lactobacillus kefiri	Kefir grains	80 % AFB1 (1 mg/L) in 24 h	Adsorption	NR	(Taheur et al., 2017)

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Table 2 (*continued*)

Microorganisms	Sources of matrices	Removal rate	Degradation substance or mechanism	Products and toxicity	Reference
Kazachstania servazzii		74 % AFB1 (1 mg/L) in 24 h			
Pichia occidentalis + *Candida sorboxylosa* + *Hanseniaspora opuntiae*	Kombucha beverage	97 % AFB1 in 7 d	Biodegradation and adsorption	The degraded products showed lower cytotoxicity than AFB1 based on cytotoxicity on Hep2 cells	(Ben Taheur et al., 2020)
Tetragenococcus halophilus CGMCC 3792	Soy sauce mash	28.31 % AFB1 (0.05 mg/L) in 1 h	Intracellular active ingredient	AFB1 was degraded to 6 non-toxic products	(Li et al., 2018a)

[α] NR: Not reported.

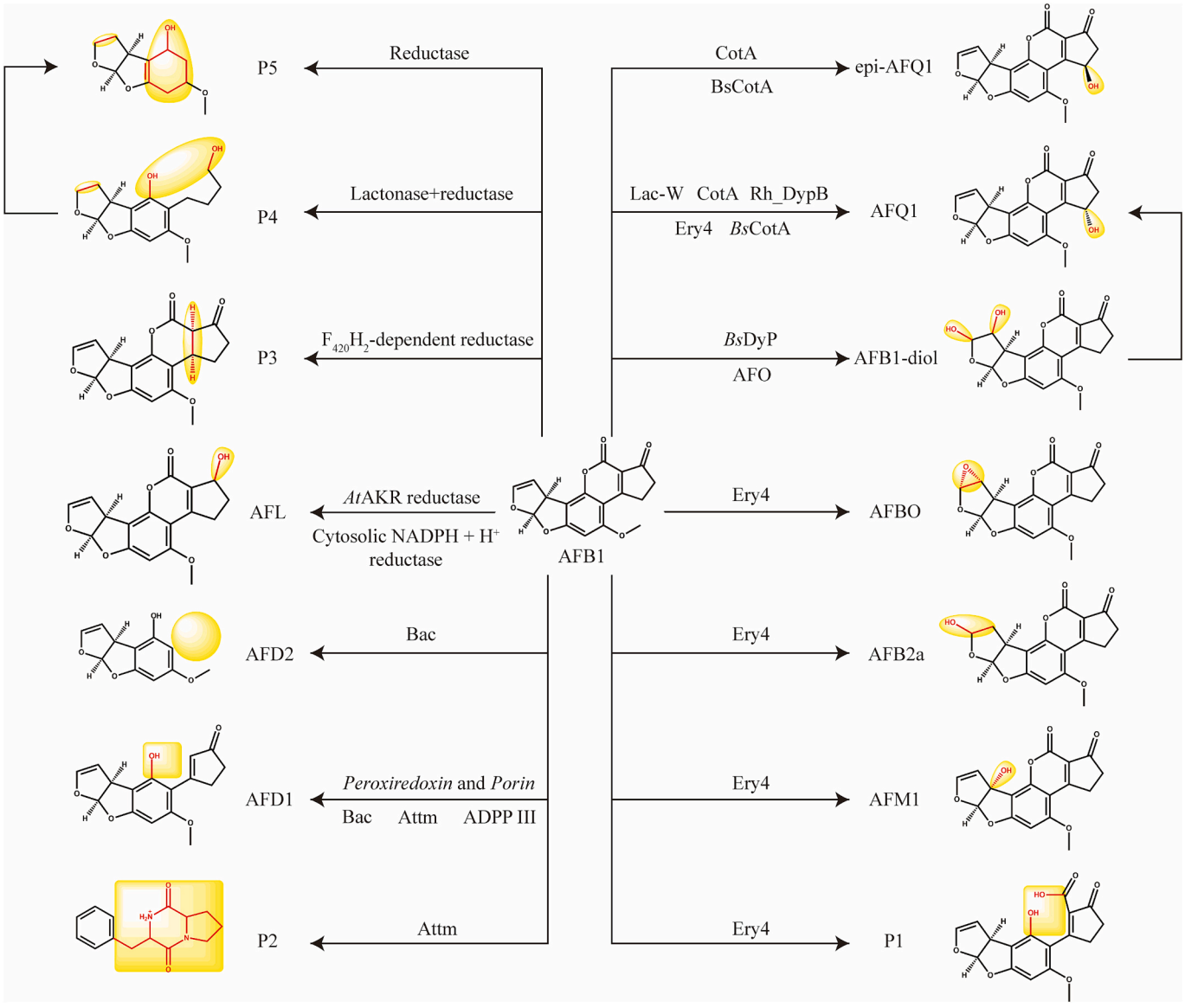

Fig. 4. Enzymatic mechanisms of AFB1-degrading oxidases, reductases, peroxidases, and lactonases. The red colour represents newly formed bonds and functional groups, whilst the yellow background represents the region where aflatoxin B1 undergoes structural changes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Adegoke et al., 2023) produce AFD1 as their primary degradation product. Zebrafish hepatotoxicity assays have demonstrated that AFD1 exhibits significantly weaker toxic effects than AFB1. Notably, *At*AKR and cytosolic NADPH+H^+ reductase convert AFB1 into aflatoxicol (AFL) (Jiang et al., 2024; Murcia & Diaz, 2020), which exhibits 18-fold less toxicity than AFB1 due to its inhibitory effect on the formation of the highly carcinogenic AFBO. The lactonase and reductase in *A. niger* destroy and hydrogenate both the furan and lactone rings in AFB1, generating the P4 and P5 products shown in Fig. 4 (Xing et al., 2017). Other enzymes such as CotA (Guo et al., 2020), *Bs*CotA (Wang et al., 2019b), Ery4 (Loi et al., 2023), and Rh_DypB (Loi et al., 2020) convert AFB1 into AFQ1 and epi-AFQ1. Recent studies on human hepatic cells

(*L*−02) have revealed that AFQ1 and epi-AFQ1 lack cytotoxic effects, attributed to their inability to form pro-mutagenic DNA adducts (Guo et al., 2020). Conversely, Ery4 laccase-mediated degradation leads to the formation of AFBO, a metabolite with heightened toxicity linked to carcinogenicity (Loi et al., 2023). This highlights that enzymatic degradation of AFs requires careful selection of enzymes that produce non-toxic byproducts, as some might yield more toxic metabolites.

4.2. Mining of AFs-degrading enzymes

To achieve efficient AFB1 degradation, researchers typically mine functional enzyme genes from toxin-degrading microorganisms or genomic databases such as NCBI and the RedoxiBase database. For example, a novel AFB1-degrading enzyme, *Ba*DyP, was recently synthesized based on sequence data from the RedoxiBase database and heterologous expressed in *Escherichia coli* (Shao et al., 2024). This

Table 3
Comparison of aflatoxin-degrading enzymes and the toxicity of their degradation products.

Enzyme	Microorganisms	Working pH	Working temperature (°C)	Degradation rate	Products and toxicity	Reference
AFO	*Armillariella tabescens*	6.0	28	100 % AFB1 (16 μmol/L) in 30 min	The enzyme treated AFB1 exhibited less mutagenic activity	(Liu et al., 2001)
AttM	*Bacillus megaterium* HNGD-A6	8.5	80	86.8 % AFB1 (2.5 μg/mL)	AFB1 was transformed into less toxic products (AFD1) by AttM	(Cheng et al., 2023)
Porin/ Peroxiredoxin	*Acinetobacter nosocomialis* Y1	9.0	80	100 % AFB1 (2.0 μg/mL) in 24 h	The cytotoxicity of product (AFD1) was significantly lower than that of AFB1	(Adegoke et al., 2023)
Lac-W	*Weizmannia coagulans* 36D1	9.0	30	88 % AFB1 (1.0 μg/mL) in 24 h	The products were AFQ1	(Hao et al., 2023b; Jia et al., 2024)
CotA	*Bacillus licheniformis* ANSB821	8.0	70	85 % AFB1 (2.0 μg/mL) in 30 min	The viability of human hepatic cells in products (AFQ1 or epi-AFQ1) was higher than that in AFB1	(Guo et al., 2020)
$F_{420}H_2$-dependent reductase	*Mycobacterium smegmatis*	7.5	[a]RT	[b]NR	NR	(Taylor et al., 2010)
MADE	*Myxococcus fulvus* ANSM068	6.0	35	96.9 % AFB1 (100 ng/mL) and 95.8 % AFM1 (100 ng/mL) in 48 h	NR	(Zhao et al., 2011)
BADE	*Bacillus shackletonii* L7	8.0	70	47.5 % AFB1 (100 ng/mL) in 3 d	genotoxicity of AFB1 was significantly reduced by BADE	(Xu et al., 2017)
TV-AFB1D	*Trametes versicolor*	NR	34	75.9 % AFB1 in 12 h	NR	(Yang et al., 2021b)
Laccase	*Trametes* sp. C30	NR	34	91 % AFB1 (0.1 μg/mL) in	The cytotoxicity and hepatotoxicity of products are significantly reduced	(Liu et al., 2021)
MnP	*Pleurotus ostreatus*	4–5	25	90 % AFB1 (1 mmol/L) in 48 h	NR	(Yehia, 2014)
Phcmnp	*Kluyveromyces lactis*	4.5	40	75.7 % AFB1 (2.0 μg/mL) in 36 h	The product AFB1–8,9-dihydrodiol was less toxic than AFB1	(Xia et al., 2022)
*Bs*CotA	*Bacillus subtilis*	7.0	60	98 % AFB1 (5.0 μg/mL) in 10 h	In hydra assay, the products showed less toxic to hydra than AFB1	(Wang et al., 2019b)
PADE	*Pseudomonas aeruginosa* M19	7.0	65	90 % AFB1 (2.5 μg/mL) in 72 h	NR	(Song et al., 2019)
Ery4	*Saccharomyces cerevisiae* ITEM 17289	5.0	25	100 % AFB1 (1 μg/mL) in 24 h	The products were identified as AFQ1, epi-AFQ1, AFM1, and AFB2a	(Loi et al., 2023)
BacC	*Bacillus subtilis* UTB1	NR	NR	NR	Bac might produce the antimicrobial di-peptide bacilysin and degrade AFB1 to AFD1 and AFD2	(Afsharmanesh et al., 2018)
GZ15	*Aspergillus niger*	NR	28	53.7 % AFB1 (354.3 ng/mL) in 9 d	The products were $C_{15}H_{20}O_5$ and $C_{11}H_{16}O_4$	(Xing et al., 2017)
JZ2				80.9 % AFB1 (354.3 ng/mL) in 9 d	The product was $C_{15}H_{20}O_5$	
*At*AKR	*Armillaria tabescens*	4.0	30	34.1 % AFB1 (100 ng/mL) in 36 h	The toxicity of product AFL was 18 times lower than that of AFB1	(Jiang et al., 2024)
ADPP III	*Aspergillus terreus* HNGD-TM15	7.0	40	97.1 % AFB1 (5 μg/mL) in 24 h	The product AFD1 showed slight hepatotoxicity	(Zhang et al., 2024b)
Rh_DypB	*Rhodococcus jostii*	6.0	25	96 % AFB1 (1.0 μg/mL) in 96 h	The product was AFQ1	(Loi et al., 2020)
*Bs*DyP	*Bacillus subtilis*	4.0	30	76.9 % AFB1 (1.0 μg/mL) in 48 h	AFB1 was degraded to less toxic product AFB1-diol	(Qin et al., 2021b)
*Ba*DyP	*Bjerkandera adusta*	4.0	30	86.6 % AFB1 (1.0 μg/mL) in 48 h	The degradation products were less toxic AFB1-diol and AFQ1	(Shao et al., 2024)
*St*MCO	*Streptomyces thermocarboxydus*	7.0	30	99.8 % AFB1 (1.0 μg/mL) in 24 h	AFB1 was degraded to less toxic product AFQ1	(Qin et al., 2021c)
Lac2	*Pleurotus pulmonarius*	5.0	25	90 % AFB1 (1.0 μg/mL) and 100 % AFM1 (0.05 μg/mL)	NR	(Loi et al., 2016)
rCuL	*Cerrena unicolor* 6884	7.0	65	NR	NR	(Zhou et al., 2022)
MSMEG_5998	*Mycobacterium smegmatis*	7.4	22	63 % AFB1 (10 μg/mL) in 4 h	MSMEG_5998 could protect the liver from AFB1 damage	(Li et al., 2019)
*Il*MnP5	*Irpex lacteus* CD2	5.0	30	94.6 % AFB1 (5 μg/mL) in 9 h	AFB1 was degrade to AFB1–8,9-epoxide	(Wang et al., 2019c)
Cytosolic NADPH + H^+ reductase	NR	Degradation conducted in in vivo chicken		NR	The product was AFL	(Murcia & Diaz, 2020)

[a] RT: Room temperature.
[b] NR: Not reported.

recombinant *Ba*DyP exhibited optimal catalytic activity at 30 °C and pH 4.0, degrading 86.6 % of AFB1 within 48 h. Degradation analysis confirmed that *Ba*DyP catalyzed the conversion of AFB1 into less toxic metabolites, specifically AFB1-diol and AFQ1. Despite these advances, mining AFs-degrading enzymes directly from microorganisms remains a well-established and prevalent approach in mycotoxin detoxification research. Cheng et al. isolated *Bacillus megaterium* HNGD-A6 from maize soil using coumarin as the sole carbon source (Cheng et al., 2023). Through genomic analysis and blastP alignment, they identified lactonase (AttM) as the key enzyme. Following heterologous expression in *Escherichia coli*, the recombinant AttM demonstrated optimal activity at pH 8.5, degrading 86.78 % of AFB1. The enzyme effectively cleaved the toxic lactone ring of AFB1, producing a significantly less toxic derivative (AFD1), thereby highlighting its potential for industrial detoxification applications in food and feed.

4.3. Working pH of different AFs-degrading enzymes

Enzymes commonly employed for AFB1 decontamination include oxidases, peroxidases, reductases, and esterase. Oxidases and peroxidases represent the most prevalent classes utilized for AFs degradation, typically functioning under acidic to neutral conditions (Table 3). Aflatoxin oxidase (AFO), one of the earliest identified degradative enzymes, achieves complete AFB1 degradation at pH 6.0 (Liu et al., 2001). Similarly, the laccase Ery4, derived from *S. cerevisiae* ITEM 17289, demonstrates nearly 100 % AFB1 degradation within 24 h at its optimal pH of 5.0 (Loi et al., 2023). The dye-degrading peroxidase *Bs*DyP efficiently degrades 86.6 % of AFB1 over 48 h at pH 4.0 (Qin et al., 2021b), whilst the multicopper oxidase *St*MCO from *Streptomyces thermocarboxydus* achieves near-complete (99.8 %) degradation within 24 h at pH 7.0 (Qin et al., 2021c). Notable exceptions include the *B. licheniformis* CotA laccase, which demonstrates optimal activity at pH 8.0 (Guo et al., 2020). Although AFB1 may serve as a natural substrate for oxidases, the supplementation of redox mediators significantly enhances degradation efficiency. For instance, the *Pleurotus pulmonarius* Lac2 laccase degraded only 23 % of AFB1 without mediators, whereas 90 % degradation was achieved upon the addition of syringaldehyde (Loi et al., 2016). Similarly, the degradation rate of *St*MCO toward AFB1 increases substantially in the presence of ferulic acid, syringaldehyde, and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as mediators (Qin et al., 2021c). These findings suggest that lignin-derived natural mediators can effectively accelerate AFB1 degradation by multicopper oxidases (MCOs), such as laccase.

4.4. Working temperature of different AFs-degrading enzymes

Most AFs-degrading enzymes exhibit optimal activity within a mesophilic temperature range (25–40 °C), corresponding to typical environmental conditions. Laccases such as TV-AFB1D (Yang et al., 2021b) and Lac2 (Loi et al., 2016) demonstrate optimal activity at 34 °C and 25 °C, respectively. The manganese peroxidases MnP from *Pleurotus ostreatus* and Phcmnp from *Kluyveromyces lactis* function optimally at 25 °C and 40 °C, respectively (Xia et al., 2022; Yehia, 2014). Similarly, peroxidases such as Rh_DypB (Loi et al., 2020) and *Ba*DyP (Shao et al., 2024) exhibit optimal AFB1-degrading activity between 25 and 30 °C. Reductases, including $F_{420}H_2$-dependent reductase and *At*AKR reductase, demonstrate effective AFB1 degradation at ambient temperature and 30 °C, respectively (Jiang et al., 2024; Li et al., 2019). Notably, several thermostable enzymes represent significant exceptions to this trend. Lactonase AttM and peroxiredoxin achieve their most efficient AFB1 degradation at 80 °C, the highest reported temperature for AFB1 degradation (Adegoke et al., 2023; Cheng et al., 2023). Additionally, CotA from *B. licheniformis* ANSB821 maintains high efficacy in AFB1 degradation at 70 °C (Guo et al., 2020). These thermal adaptations highlight the remarkable diversity of enzymatic strategies, enabling tailored applications across various industrial settings.

4.5. Comparison of AFs-degrading enzymes efficiency in different fields

The mitigation of AFs contamination in food or feed matrices such as milk, beer, peanut, and corn has been achieved through enzymatic degradation. Recombinant superoxide dismutase (rSOD) derived from *B. pumilus* E-1-1-1 degraded 15.19 % and 26.03 % of AFM1 in beer and milk, respectively, during 24 h incubation at 40 °C (Liu et al., 2024). Toxicity assays using Hep-G2 cells revealed that rSOD-treated AFM1 samples increased cell survival rates by 1.6-fold compared to untreated controls, with this reduction in toxicity attributed to hydrogen bond-mediated detoxification. Similarly, manganese peroxidase (rPODs) from *B. pumilus* achieved 25.6 % reduction of AFM1 in milk and 18.2 % in beer when incubated at 30 °C for 24 h, enhancing Hep-G2 cell viability by 1.4-fold. Recent studies have highlighted the potential of Ery4 laccase in feed detoxification (Loi et al., 2023), demonstrating 26 % degradation efficiency when applied to AFB1-contaminated corn under optimized conditions. Despite these advances, the modest degradation efficiency (generally <30 %) limits industrial scalability. Current AFs-degrading enzymes face limitations in industrial application due to inefficiency and low stability.

5. Recent advances on enzyme-based AFs mitigation

5.1. Machine-learning based identification for novel mycotoxin degrading enzyme

Moreover, the ToxinDB database represents another critical advancement in predicting toxin degradation mechanisms by analyzing structural inputs to infer potential enzymatic pathways (Zhang et al., 2021). Its comprehensive architecture incorporates over 8000 biotransformation reaction rules derived from more than 300,000 biochemical records, enabling users to simulate various enzymatic processes including oxidation, reduction, and hydrolysis. When AFB1 is input, a rule-based algorithm predicts fewer toxic metabolites, thereby guiding enzyme candidate identification. Furthermore, a positive unlabeled learning-based enzyme promiscuity prediction (PU-EPP) program, trained on enzyme sequence-structure databases, enables high-throughput screening for mycotoxin-degrading enzymes (Zhang et al., 2024c). PU-EPP employs graph neural networks (GNNs) to capture the structural and chemical properties of substrates, whilst a continuous bag-of-words (CBOW) approach represents enzyme sequences. The substrate-enzyme features and critical interaction sites of enzymes are integrated through a multi-head attention mechanism. The machine learning-driven PU-EPP method has successfully identified 15 novel enzymes targeting ochratoxin A and ZEN, with six candidates demonstrating the ability to degrade over 90 % of these toxins within 3 h. The underlying machine-learning framework is inherently substrate-agnostic and can be readily extended to AFs. By training on structural features common to bisfuranocoumarin cores, PU-EPP can predict enzyme candidates with high affinity for AFs. Of course, a further experimental investigation is essentially required to fully establish the applicability of machine learning-based frameworks in identifying effective AFs-degrading enzymes.

5.2. Enzyme modification for higher activity and stability of AFs-degrading enzyme

In practical food and feed applications, AFs-degrading enzymes require further optimization to meet industrial requirements. The $F_{420}H_2$-dependent reductase, when fused with thioredoxin (Trx) at its N-terminal region, demonstrated a 2-fold enhancement in AFB1 degradation compared to the unmodified enzyme (Li et al., 2019). Specifically, the Trx-linked enzyme achieved 63 % degradation of AFB1, whilst the unmodified variant exhibited only 31 % removal under identical experimental conditions. Computational strategies, including in silico molecular docking and rational protein engineering, facilitate precise

enzyme modification. For instance, researchers employed the Discovery Studio (DS) program for homology modelling and virtual mutation of laccase r*CuL* from *C. unicolor* (Zhou et al., 2022). Virtual mutations of Asn336, Asp207, Val391, and Thr165 revealed the critical role of hydrogen bonding in AFB1-r*CuL* interactions. In another study, molecular dynamics (MD) simulations between enzyme (AFO) and substrate (AFB1) using the Amber16 program package demonstrated that AFB1 is not the most suitable substrate for AFO due to negatively charged regions in the active site that are incompatible with neutral AFB1 (Tomin & Tomić, 2019). Furthermore, Yang et al. successfully implemented rational design of AFB1-degrading enzymes through molecular docking and site-directed mutagenesis, identifying key residues E436 and H554 that hindered substrate binding (Yang et al., 2021a). By replacing these residues with alanine (E436A/H554A double mutant), they reduced steric hindrance and achieved a more favorable substrate orientation, resulting in a remarkable 1.84-fold increase in enzymatic activity compared to the wild-type under optimal conditions. Moreover, Jia et al. generated thermostable mutants of MADE through error-prone PCR, establishing a library of 5000 variants with initial high-throughput screening based on coumarin as the sole carbon source (Jia et al., 2023). Three mutants exhibited significantly enhanced T_{50}^{60} values (the temperature at which enzyme activity is reduced by 50 % after 1 h at 60 °C), with increases of 16.5 °C, 6.5 °C, and 9.8 °C compared to the wild-type MADE. Additionally, two mutants demonstrated approximately 80 % increased catalytic efficiency compared to the wild-type.

5.3. Perspectives and future outlook

The advances in enhancing enzyme-based AFs detoxification suggest the potential for an integrated approach combining rapid AFB1 detection, machine learning-driven enzyme identification, enzyme-mediated degradation, and computer-aided modification. Emerging platforms, such as smartphone-based colorimetric systems, enable on-site quantification of AFB1. For example, Zhou et al. developed a sophisticated smartphone-based detection platform by synthesizing SSM/COF-Apt_1 (Zhou et al., 2024). They immobilized and conjugated Au@Ir nanoparticles with Apt_2 aptamers (Au@Ir-Apt_2) as molecular probes. The SSM/COF-Apt_1 and Au@Ir-Apt_2 effectively capture AFB1 to form a sandwich complex (SSM/COF-Apt_1-AFB1-Apt_2-Au@Ir), which subsequently triggers a TMB/H_2O_2 colorimetric reaction in the presence of AFB1. The colorimetric signal is transferred to a smartphone and converted into AFB1 concentration data via a customized RGB analysis algorithm, as shown in Fig. 5. This innovative platform achieves a detection limit of 0.045 ng/mL for AFB1, combining portability, real-time analysis, and high sensitivity. Advancements in bioinformatics further enable the prediction of degradation pathways through machine learning-driven analysis of enzyme-substrate interactions. Furthermore, computational approaches, such as molecular docking, molecular dynamics simulations, and in silico mutational analysis, have proven effective in enhancing the catalytic activity and stability of AFs-degrading enzymes. The integration of these innovations with robust monitoring technologies establishes a computer-aided biodefence framework that dynamically adapts to AFs contamination risks, ensuring comprehensive management throughout the production chain.

6. Conclusions

Biological strategies involving microorganisms and specialized enzymes offer promising detoxification solutions. Advances in intelligent detection, computer-assisted enzyme discovery, and computational

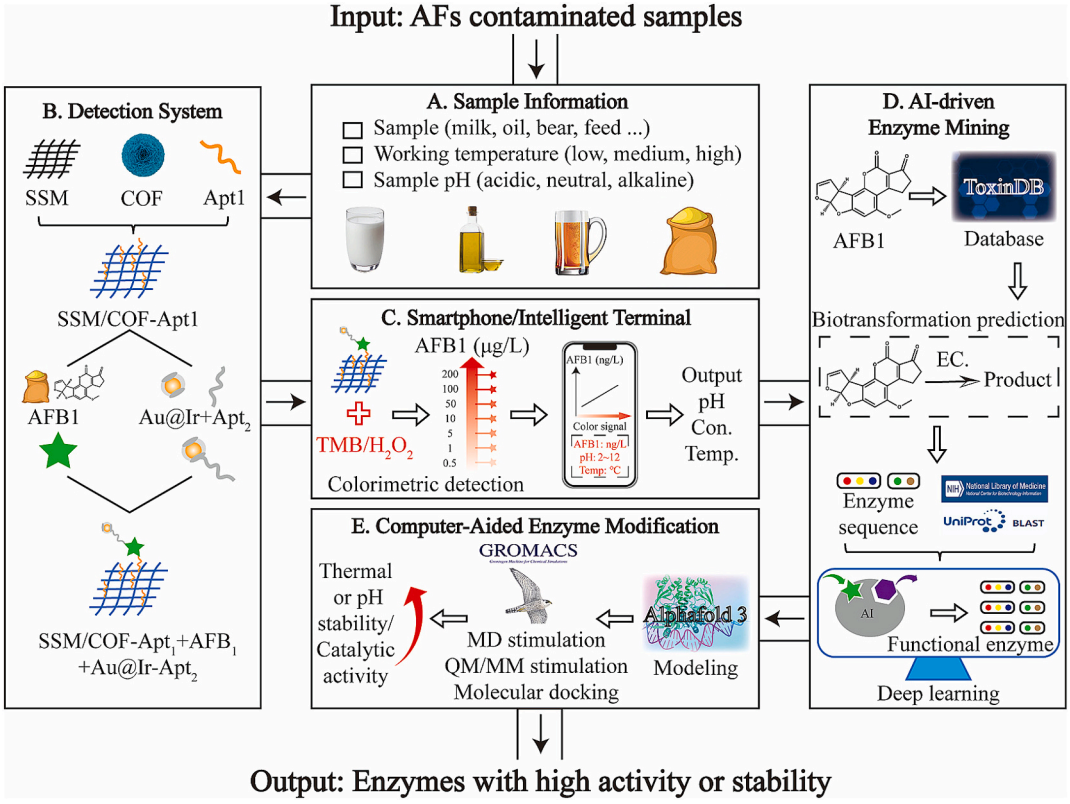

Fig. 5. The proposed computer-aided framework for AFB1 mitigation in food and feed supply chain. (A) Sample information of AFB contamination, sample pH, and working temperature. (B) A biosensor for real-time AFB1 detection comprises SSM/COF-Apt_1 and Au@Ir-Apt_2, which capture AFB1 to form a sandwich complex, SSM/COF-Apt_1-AFB1-Apt_2-Au@Ir. The sandwich complex reacts with TMB/H_2O_2, triggering a colorimetric reaction. (C) The intelligent terminal then displays the AFB1 concentration based on the colour change induced by the interaction between the sandwich complex and TMB/H_2O_2. (D) computer-aided AFs-degrading enzyme identification. (E) Computer-aided enzyme modification for enhancement of enzyme stability and activity.

design have enhanced catalytic efficiency and real-time monitoring. However, limited enzyme stability, suboptimal activity under processing conditions, and the safety of degradation products hinder large-scale application. In the future, efforts should focus on mining and engineering robust, food-grade enzymes using advanced computer-assisted enzyme discovery tools and computer-aided enzyme modification strategies. Moreover, integrating these technologies with upstream intelligent AFs detection tool will enable dynamic and precise control of AFs contamination.

CRediT authorship contribution statement

Binbin Ouyang: Writing – original draft, Investigation. **Wei Xu:** Writing – review & editing. **Dawei Ni:** Formal analysis. **Wenli Zhang:** Resources. **Junmei Ding:** Software. **Wanmeng Mu:** Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Adebo, O. A., Njobeh, P. B., & Mavumengwana, V. (2016). Degradation and detoxification of AFB1 by *Staphylococcus warneri*, *Sporosarcina* sp. and *Lysinibacillus fusiformis*. *Food Control, 68*, 92–96. https://doi.org/10.1016/j.foodcont.2016.03.021

Adebo, O. A., Njobeh, P. B., Sidu, S., Tlou, M. G., & Mavumengwana, V. (2016). Aflatoxin B1 degradation by liquid cultures and lysates of three bacterial strains. *International Journal of Food Microbiology, 233*, 11–19. https://doi.org/10.1016/j.ijfoodmicro.2016.06.007

Adegoke, T. V., Yang, B., Tian, X., Yang, S., Gao, Y., Ma, J., … Xing, F. (2023). Simultaneous degradation of aflatoxin B1 and zearalenone by Porin and Peroxiredoxin enzymes cloned from *Acinetobacter nosocomialis* Y1. *Journal of Hazardous Materials, 459*, Article 132105. https://doi.org/10.1016/j.jhazmat.2023.132105

Afsharmanesh, H., Perez-Garcia, A., Zeriouh, H., Ahmadzadeh, M., & Romero, D. (2018). Aflatoxin degradation by *Bacillus subtilis* UTB1 is based on production of an oxidoreductase involved in bacilysin biosynthesis. *Food Control, 94*, 48–55. https://doi.org/10.1016/j.foodcont.2018.03.002

Ali, S., Hassan, M., Essam, T., Ibrahim, M. A., & Al-Amry, K. (2021). Biodegradation of aflatoxin by bacterial species isolated from poultry farms. *Toxicon, 195*, 7–16. https://doi.org/10.1016/j.toxicon.2021.02.005

Al-Saadi, H. A., Al-Sadi, A. M., Al-Wahaibi, A., Al-Raeesi, A., Al-Kindi, M., Pandian, S. B. S., … Velazhahan, R. (2024). Rice weevil (*Sitophilus oryzae* L.) gut bacteria inhibit growth of *aspergillus flavus* and degrade aflatoxin B1. *Journal of Fungi, 10*(6), 377. https://doi.org/10.3390/jof10060377

Ben Taheur, F., Mansour, C., Ben Jeddou, K., Machreki, Y., Kouidhi, B., Abdulhakim, J. A., & Chaieb, K. (2020). Aflatoxin B1 degradation by microorganisms isolated from Kombucha culture. *Toxicon, 179*, 76–83. https://doi.org/10.1016/j.toxicon.2020.03.004

Benkerroum, N. (2020). Chronic and acute toxicities of aflatoxins: Mechanisms of action. *International Journal of Environmental Research and Public Health, 17*(2), 423. https://doi.org/10.3390/ijerph17020423

Bi, Z., Yi, X., Yang, S., Yu, Z., & Li, L. (2023). Food aflatoxin exposure assessment in Sichuan Province, China. *Mycotoxin Research, 39*(3), 261–269. https://doi.org/10.1007/s12550-023-00488-0

Branà, M. T., Cimmarusti, M. T., Haidukowski, M., Logrieco, A. F., & Altomare, C. (2017). Bioremediation of aflatoxin B1-contaminated maize by king oyster mushroom (*Pleurotus eryngii*). *PLoS One, 12*(8), Article e0182574. https://doi.org/10.1371/journal.pone.0182574

Caceres, I., Al Khoury, A., El Khoury, R., Lorber, S., Oswald, I., El Khoury, A., Atoui, A., Puel, O., & Bailly, J.-D. (2020). Aflatoxin biosynthesis and genetic regulation: A review. *Toxins, 12*(3), 150. https://doi.org/10.3390/toxins12030150

Cai, M., Qian, Y., Chen, N., Ling, T., Wang, J., Jiang, H., Wang, X., Qi, K., & Zhou, Y. (2020). Detoxification of aflatoxin B1 by *Stenotrophomonas* sp. CW117 and characterization the thermophilic degradation process. *Environmental Pollution, 261*, Article 114178. https://doi.org/10.1016/j.envpol.2020.114178

Cao, W. Y., Yu, P., Yang, K. P., & Cao, D. L. (2022). Aflatoxin B1: Metabolism, toxicology, and its involvement in oxidative stress and cancer development. *Toxicology Mechanisms and Methods, 32*(6), 395–419. https://doi.org/10.1080/15376516.2021.2021339

Cheng, S., Feng, X., Liu, G., Zhao, N., Liu, J., Zhang, Z., Yang, N., Zhou, L., Pang, M., Tang, B., Dong, J., Zhao, B., & Liu, Y. (2022). Natural occurrence of mycotoxins in maize in North China. *Toxins, 14*(8), 521. https://doi.org/10.3390/toxins14080521

Cheng, S., Wu, T., Zhang, H., Sun, Z., Mwabulili, F., Xie, Y., Sun, S., Ma, W., Li, Q., Yang, Y., Wu, X., & Jia, H. (2023). Mining lactonase gene from aflatoxin B1-degrading strain *Bacillus megaterium* and degrading properties of the recombinant enzyme. *Journal of Agricultural and Food Chemistry, 71*(51), 20762–20771. https://doi.org/10.1021/acs.jafc.3c05725

Chu, X., Wang, W., Yoon, S.-C., Ni, X., & Heitschmidt, G. W. (2017). Detection of aflatoxin B1 (AFB1) in individual maize kernels using short wave infrared (SWIR) hyperspectral imaging. *Biosystems Engineering, 157*, 13–23. https://doi.org/10.1016/j.biosystemseng.2017.02.005

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

Cui, P., Yan, H., Granato, D., Ho, C.-T., Ye, Z., Wang, Y., Zhang, L., & Zhou, Y. (2020). Quantitative analysis and dietary risk assessment of aflatoxins in Chinese post-fermented dark tea. *Food and Chemical Toxicology, 146*, 111830. https://doi.org/10.1016/j.fct.2020.111830

Das, A., Bhattacharya, S., Palaniswamy, M., & Angayarkanni, J. (2014). Biodegradation of aflatoxin B1 in contaminated rice straw by *Pleurotus ostreatus* MTCC 142 and *Pleurotus ostreatus* GHBBF10 in the presence of metal salts and surfactants. *World Journal of Microbiology and Biotechnology, 30*(8), 2315–2324. https://doi.org/10.1007/s11274-014-1657-5

Elaasser, M. M., & El Kassas, R. A. (2011). Detoxification of aflatoxin B1 by certain bacterial species isolated from Egyptian soil. *World Mycotoxin Journal, 4*(2), 169–176. https://doi.org/10.3920/wmj2010.1262

El-Deeb, B., Altalhi, A., Khiralla, G., Hassan, S., & Gherbawy, Y. (2013). Isolation and characterization of endophytic *Bacilli bacterium* from maize grains able to detoxify aflatoxin B1. *Food Biotechnology, 27*(3), 199–212. https://doi.org/10.1080/08905436.2013.811083

Elsanhoty, R. M., Al-Turki, I. A., & Ramadan, M. F. (2016). Application of lactic acid bacteria in removing heavy metals and aflatoxin B1 from contaminated water. *Water Science and Technology, 74*(3), 625–638. https://doi.org/10.2166/wst.2016.255

Eshelli, M., Harvey, L., Edrada-Ebel, R., & McNeil, B. (2015). Metabolomics of the biodegradation process of aflatoxin B1 by Actinomycetes at an initial pH of 6.0. *Toxins, 7*(2), 439–456. https://doi.org/10.3390/toxins7020439

Fang, Q., Du, M., Chen, J., Liu, T., Zheng, Y., Liao, Z., … Wang, J. (2020). Degradation and detoxification of aflatoxin B1 by tea-derived *aspergillus Niger* RAF106. *Toxins, 12*(12). https://doi.org/10.3390/toxins12120777

Farzaneh, M., Shi, Z.-Q., Ghassempour, A., Sedaghat, N., Ahmadzadeh, M., Mirabolfathy, M., & Javan-Nikkhah, M. (2012). Aflatoxin B1 degradation by *Bacillus subtilis* UTBSP1 isolated from pistachio nuts of Iran. *Food Control, 23*(1), 100–106. https://doi.org/10.1016/j.foodcont.2011.06.018

Feng, J., Cao, L., Du, X., Zhang, Y., Cong, Y., He, J., & Zhang, W. (2024). Biological detoxification of aflatoxin B1 by *enterococcus faecium* HB2-2. *Foods, 13*(12), 1887. https://doi.org/10.3390/foods13121887

China Food and Drug Administration. (2017). National food safety standard. GB 2761-2017.

García-Béjar, B., Arévalo-Villena, M., Guisantes-Batan, E., Rodríguez-Flores, J., & Briones, A. (2020). Study of the bioremediatory capacity of wild yeasts. *Scientific Reports, 10*(1), 11265. https://doi.org/10.1038/s41598-020-68154-4

González Pereyra, M. L., Martínez, M. P., & Cavaglieri, L. R. (2019). Presence of aiiA homologue genes encoding for N-acyl homoserine lactone-degrading enzyme in aflatoxin B1-decontaminating *Bacillus* strains with potential use as feed additives. *Food and Chemical Toxicology, 124*, 316–323. https://doi.org/10.1016/j.fct.2018.12.016

Gu, X., Sun, J., Cui, Y., Wang, X., & Sang, Y. (2019). Biological degradation of aflatoxin M1 by *Bacillus pumilus* E-1-1-1. *MicrobiologyOpen, 8*(3), Article e00663. https://doi.org/10.1002/mbo3.663

Guan, S., Ji, C., Zhou, T., Li, J., Ma, Q., & Niu, T. (2008). Aflatoxin B1 degradation by *Stenotrophomonas maltophilia* and other microbes selected using coumarin medium. *International Journal of Molecular Sciences, 9*(8), 1489–1503. https://doi.org/10.3390/ijms9081489

Guo, C., Fan, L., Yang, Q., Ning, M., Zhang, B., & Ren, X. (2024). Characterization and mechanism of simultaneous degradation of aflatoxin B1 and zearalenone by an edible fungus of *Agrocybe cylindracea* GC-Ac2. *Frontiers in Microbiology, 15*, 1292824. https://doi.org/10.3389/fmicb.2024.1292824

Guo, J., Zhang, H., Zhao, Y., Hao, X., Liu, Y., Li, S., & Wu, R. (2024). Identification of a novel aflatoxin B1-degrading strain, *Bacillus halotolerans* DDC-4, and its response mechanisms to aflatoxin B1. *Toxins, 16*(6), 256. https://doi.org/10.3390/toxins16060256

Guo, L., Wang, Y., Fei, P., Liu, J., & Ren, D. (2019). A survey on the aflatoxin M1 occurrence in raw milk and dairy products from water buffalo in South China. *Food Control, 105*, 159–163. https://doi.org/10.1016/j.foodcont.2019.05.033

Guo, Y., Qin, X., Tang, Y., Ma, Q., Zhang, J., & Zhao, L. (2020). CotA laccase, a novel aflatoxin oxidase from *Bacillus licheniformis*, transforms aflatoxin B1 to aflatoxin Q1 and epi-aflatoxin Q1. *Food Chemistry, 325*, Article 126877. https://doi.org/10.1016/j.foodchem.2020.126877

Halttunen, T., Collado, M. C., El-Nezami, H., Meriluoto, J., & Salminen, S. (2008). Combining strains of lactic acid bacteria may reduce their toxin and heavy metal removal efficiency from aqueous solution. *Letters in Applied Microbiology, 46*(2), 160–165. https://doi.org/10.1111/j.1472-765X.2007.02276.x

Hao, W., Guan, S., Li, A., Wang, J., An, G., Hofstetter, U., & Schatzmayr, G. (2023). Mycotoxin occurrence in deeds and raw materials in China: A five-year investigation. *Toxins, 15*(1), 63. https://doi.org/10.3390/toxins15010063

Hao, W.-B., Gu, X., Yu, X., Zhao, Y., Li, C., Jia, M., & Du, X.-D. (2023). Laccase lac-W detoxifies aflatoxin B1 and degrades five other major mycotoxins in the absence of redox mediators. *Environmental Pollution, 338*, Article 122581. https://doi.org/10.1016/j.envpol.2023.122581

Harkai, P., Szabó, I., Cserháti, M., Krifaton, C., Risa, A., Radó, J., Balázs, A., Berta, K., & Kriszt, B. (2016). Biodegradation of aflatoxin-B1 and zearalenone by *Streptomyces* sp. collection. *International Biodeterioration & Biodegradation, 108*, 48–56. https://doi.org/10.1016/j.ibiod.2015.12.007

Jackson, L. W., & Pryor, B. M. (2017). Degradation of aflatoxin B1 from naturally contaminated maize using the edible fungus *Pleurotus ostreatus*. *AMB Express, 7*(1), 110. https://doi.org/10.1186/s13568-017-0415-0

Jallow, A., Xie, H., Tang, X., Qi, Z., & Li, P. (2021). Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Comprehensive Reviews in Food Science and Food Safety, 20*(3), 2332–2381. https://doi.org/10.1111/1541-4337.12734

Jia, M., Yu, X., Xu, K., Gu, X., Harmer, N. J., Zhao, Y., … Hao, W. (2024). The high-efficiency degradation of multiple mycotoxins by lac-W laccase in the presence of mediators. *Toxins, 16*(11), 477. https://doi.org/10.3390/toxins16110477

Jia, R., Tian, S., Yang, Z., Sadiq, F. A., Wang, L., Lu, S., … Li, J. (2023). Tuning thermostability and catalytic efficiency of aflatoxin-degrading enzyme by error-prone PCR. *Applied Microbiology and Biotechnology, 107*(15), 4833–4843. https://doi.org/10.1007/s00253-023-12610-4

Jiang, T., Li, F., Li, F., Xie, C., Liu, D., & Yao, D. (2024). Degradation of aflatoxin B1 by the *Armillariella tabescens*-derived aldo-keto reductase *At*AKR. *Food Bioscience, 58*, Article 103768. https://doi.org/10.1016/j.fbio.2024.103768

Krifaton, C., Kriszt, B., Szoboszlay, S., Cserháti, M., Szűcs, Á., & Kukolya, J. (2011). Analysis of aflatoxin-B1-degrading microbes by use of a combined toxicity-profiling method. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 726*(1), 1–7. https://doi.org/10.1016/j.mrgentox.2011.07.011

Kumar, V., Bahuguna, A., Lee, J. S., Sood, A., Han, S. S., Chun, H. S., & Kim, M. (2023). Degradation mechanism of aflatoxin B1 and aflatoxin G1 by salt tolerant *Bacillus albus* YUN5 isolated from "doenjang", a traditional Korean food. *Food Research International, 165*, Article 112479. https://doi.org/10.1016/j.foodres.2023.112479

Li, A., Hao, W., Guan, S., Wang, J., & An, G. (2022). Mycotoxin contamination in feeds and feed materials in China in year 2020. *Frontiers in Veterinary Science, 9*, 1016528. https://doi.org/10.3389/fvets.2022.1016528

Li, C.-H., Li, W.-Y., Hsu, I. N., Liao, Y.-Y., Yang, C.-Y., Taylor, M. C., & Tsai, R.-T. (2019). Recombinant aflatoxin-degrading $F_{420}H_2$-dependent reductase from *mycobacterium smegmatis* protects mammalian cells from aflatoxin toxicity. *Toxins, 11*(5), 259. https://doi.org/10.3390/toxins11050259

Li, F., Zhao, X., Jiao, Y., Duan, X., Yu, L., Zheng, F., … Zhou, J. (2023). Exposure assessment of aflatoxins and zearalenone in edible vegetable oils in Shandong, China: Health risks posed by mycotoxin immunotoxicity and reproductive toxicity in children. *Environmental Science and Pollution Research, 30*(2), 3743–3758. https://doi.org/10.1007/s11356-022-22385-2

Li, J., Huang, J., Jin, Y., Wu, C., Shen, D., Zhang, S., & Zhou, R. (2018a). Aflatoxin B1 degradation by salt tolerant *Tetragenococcus halophilus* CGMCC 3792. *Food and Chemical Toxicology, 121*, 430–436. https://doi.org/10.1016/j.fct.2018.08.063

Li, J., Huang, J., Jin, Y., Wu, C., Shen, D., Zhang, S., & Zhou, R. (2018b). Mechanism and kinetics of degrading aflatoxin B1 by salt tolerant *Candida versatilis* CGMCC 3790. *Journal of Hazardous Materials, 359*, 382–387. https://doi.org/10.1016/j.jhazmat.2018.05.053

Liu, D.-L., Yao, D.-S., Liang, Y.-Q., Zhou, T.-H., Song, Y.-P., Zhao, L., & Ma, L. (2001). Production, purification, and characterization of an intracellular aflatoxin-detoxifizyme from *Armillariella tabescens* (E-20). *Food and Chemical Toxicology, 39*(5), 461–466. https://doi.org/10.1016/S0278-6915(00)00161-7

Liu, X., Zhao, F., Wang, X., & Sang, Y. (2024). Superoxide dismutase, a novel aflatoxin oxidase from *Bacillus pumilus* E-1-1-1: Study on the degradation mechanism of aflatoxin M1 and its application in milk and beer. *Food Control, 161*, Article 110372. https://doi.org/10.1016/j.foodcont.2024.110372

Liu, Y., Mao, H., Yohannes, K. W., Wan, Z., Cao, Y., Tron, T., … Wang, J. (2021). Degradation of aflatoxin B1 by a recombinant laccase from *Trametes* sp. C30 expressed in *Saccharomyces cerevisiae*: A mechanism assessment study *in vitro* and *in vivo*. *Food Research International, 145*, Article 110418. https://doi.org/10.1016/j.foodres.2021.110418

Loi, M., De Leonardis, S., Ciasca, B., Paciolla, C., Mulè, G., & Haidukowski, M. (2023). Aflatoxin B1 degradation by Ery4 laccase: From *in vitro* to contaminated corn. *Toxins, 15*(5), 310. https://doi.org/10.3390/toxins15050310

Loi, M., Fanelli, F., Zucca, P., Liuzzi, V. C., Quintieri, L., Cimmarusti, M. T., … Mulè, G. (2016). Aflatoxin B1 and M1 degradation by Lac2 from *Pleurotus pulmonarius* and redox mediators. *Toxins, 8*(9), 245. https://doi.org/10.3390/toxins8090245

Loi, M., Renaud, J. B., Rosini, E., Pollegioni, L., Vignali, E., Haidukowski, M., … Mulè, G. (2020). Enzymatic transformation of aflatoxin B1 by Rh_DypB peroxidase and characterization of the reaction products. *Chemosphere, 250*, Article 126296. https://doi.org/10.1016/j.chemosphere.2020.126296

Luo, X., Wang, R., Wang, L., Li, Y., Wang, Y., & Chen, Z. (2014). Detoxification of aflatoxin in corn flour by ozone. *Journal of the Science of Food and Agriculture, 94*(11), 2253–2258. https://doi.org/10.1002/jsfa.6550

Moustafa, M., Taha, T., Elnouby, M., El-Deeb, N., Hamad, G., Abusaied, M. A., & Alrumman, S. (2017). Potential detoxification of aflatoxin B2 using *Kluyveromyces lactis* and *Saccharomyces cerevisiae* integrated nanofibers. *BIOCELL, 41*, 2–3. https://doi.org/10.32604/biocell.2017.41.067

Murcia, H. W., & Diaz, G. J. (2020). *In vitro* hepatic aflatoxicol production is related to a higher resistance to aflatoxin B1 in poultry. *Scientific Reports, 10*(1), 5508. https://doi.org/10.1038/s41598-020-62415-y

Ning, M., Zhang, S., Xie, Y., Wang, W., & Gao, Y. (2019). Aflatoxin B1 removal by three bacterial strains and optimization of fermentation process parameters. *Biotechnology and Applied Biochemistry, 66*(6), 930–938. https://doi.org/10.1002/bab.1807

Obrian, G. R., Georgianna, D. R., Wilkinson, J. R., Yu, J., Abbas, H. K., Bhatnagar, D., … Payne, G. A. (2007). The effect of elevated temperature on gene transcription and aflatoxin biosynthesis. *Mycologia, 99*(2), 232–239. https://doi.org/10.1080/15572536.2007.11832583

Petchkongkaew, A., Taillandier, P., Gasaluck, P., & Lebrihi, A. (2008). Isolation of *Bacillus* spp. from Thai fermented soybean (Thua-nao): Screening for aflatoxin B1 and ochratoxin a detoxification. *Journal of Applied Microbiology, 104*(5), 1495–1502. https://doi.org/10.1111/j.1365-2672.2007.03700.x

Qin, M., Liang, J., Yang, D., Yang, X., Cao, P., Wang, X., Ma, N., & Zhang, L. (2021). Spatial analysis of dietary exposure of aflatoxins in peanuts and peanut oil in different areas of China. *Food Research International, 140*, Article 109899. https://doi.org/10.1016/j.foodres.2020.109899

Qin, X., Su, X., Tu, T., Zhang, J., Wang, X., Wang, Y., Wang, Y., Bai, Y., Yao, B., Luo, H., & Huang, H. (2021). Enzymatic degradation of multiple major mycotoxins by dye-decolorizing peroxidase from *Bacillus subtilis*. *Toxins, 13*(6), 429. https://doi.org/10.3390/toxins13060429

Qin, X., Xin, Y., Zou, J., Su, X., Wang, X., Wang, Y., Zhang, J., Tu, T., Yao, B., Luo, H., & Huang, H. (2021). Efficient degradation of aflatoxin B1 and zearalenone by laccase-like multicopper oxidase from *Streptomyces thermocarboxydus* in the presence of mediators. *Toxins, 13*(11), 754. https://doi.org/10.3390/toxins13110754

Qiu, T., Wang, H., Yang, Y., Yu, J., Ji, J., Sun, J., … Sun, X. (2021). Exploration of biodegradation mechanism by AFB1-degrading strain *aspergillus Niger* FS10 and its metabolic feedback. *Food Control, 121*, Article 107609. https://doi.org/10.1016/j.foodcont.2020.107609

Raksha Rao, K., Vipin, A. V., Hariprasad, P., Anu Appaiah, K. A., & Venkateswaran, G. (2017). Biological detoxification of aflatoxin B1 by *Bacillus licheniformis* CFR1. *Food Control, 71*, 234–241. https://doi.org/10.1016/j.foodcont.2016.06.040

Reverberi, M., Zjalic, S., Ricelli, A., Fabbri, A. A., & Fanelli, C. (2006). Oxidant/antioxidant balance in *aspergillus parasiticus* affects aflatoxin biosynthesis. *Mycotoxin Research, 22*(1), 39–47. https://doi.org/10.1007/BF02954556

Risa, A., Krifaton, C., Kukolya, J., Kriszt, B., Cserháti, M., & Táncsics, A. (2018). Aflatoxin B1 and zearalenone-detoxifying profile of *Rhodococcus* type strains. *Current Microbiology, 75*(7), 907–917. https://doi.org/10.1007/s00284-018-1465-5

Samuel, M. S., Sivaramakrishna, A., & Mehta, A. (2014). Degradation and detoxification of aflatoxin B1 by *pseudomonas putida*. *International Biodeterioration & Biodegradation, 86*, 202–209. https://doi.org/10.1016/j.ibiod.2013.08.026

Shao, H., Su, X., Wang, Y., Zhang, J., Tu, T., Wang, X., Huang, H., Yao, B., Luo, H., & Qin, X. (2024). Oxidative degradation and detoxification of multiple mycotoxins using a dye-decolorizing peroxidase from the white-rot fungus *Bjerkandera adusta*. *LWT, 206*, Article 116597. https://doi.org/10.1016/j.lwt.2024.116597

Shetty, P. H., Hald, B., & Jespersen, L. (2007). Surface binding of aflatoxin B1 by *Saccharomyces cerevisiae* strains with potential decontaminating abilities in indigenous fermented foods. *International Journal of Food Microbiology, 113*(1), 41–46. https://doi.org/10.1016/j.ijfoodmicro.2006.07.013

Shi, H., Chang, G., Zhang, Y., Zhao, Y., Wang, H., Zhang, J., & Zhu, J. (2024). Biodegradation characteristics and mechanism of aflatoxin B1 by *Bacillus amyloliquefaciens* from enzymatic and multiomics perspectives. *Journal of Agricultural and Food Chemistry, 72*(28), 15841–15853. https://doi.org/10.1021/acs.jafc.4c04055

Shu, X., Wang, Y., Zhou, Q., Li, M., Hu, H., Ma, Y., Chen, X., Ni, J., Zhao, W., Huang, S., & Wu, L. (2018). Biological degradation of aflatoxin B1 by cell-free extracts of *Bacillus velezensis* DY3108 with broad pH stability and excellent thermostability. *Toxins, 10*(8), 330. https://doi.org/10.3390/toxins10080330

Singh, J., & Mehta, A. (2019). Protein-mediated degradation of aflatoxin B1 by *pseudomonas putida*. *Brazilian Journal of Microbiology, 50*(4), 1031–1039. https://doi.org/10.1007/s42770-019-00134-x

Song, J., Zhang, S., Xie, Y., & Li, Q. (2019). Purification and characteristics of an aflatoxin B1 degradation enzyme isolated from *Pseudomonas aeruginosa*. *FEMS Microbiology Letters, 366*(5), fnz034. https://doi.org/10.1093/femsle/fnz034

Sun, X. D., Su, P., & Shan, H. (2017). Mycotoxin contamination of rice in China. *Journal of Food Science, 82*(3), 573–584. https://doi.org/10.1111/1750-3841.13631

Taheur, F. B., Fedhila, K., Chaieb, K., Kouidhi, B., Bakhrouf, A., & Abrunhosa, L. (2017). Adsorption of aflatoxin B1, zearalenone and ochratoxin a by microorganisms isolated from kefir grains. *International Journal of Food Microbiology, 251*, 1–7. https://doi.org/10.1016/j.ijfoodmicro.2017.03.021

Taylor, M. C., Jackson, C. J., Tattersall, D. B., French, N., Peat, T. S., Newman, J., & Oakeshott, J. G. (2010). Identification and characterization of two families of $F_{420}H_2$-dependent reductases from *mycobacteria* that catalyse aflatoxin degradation. *Molecular Microbiology, 78*(3), 561–575. https://doi.org/10.1111/j.1365-2958.2010.07356.x

Tomin, M., & Tomić, S. (2019). Oxidase or peptidase? A computational insight into a putative aflatoxin oxidase from *Armillariella tabescens*. *Proteins, 87*(5), 390–400. https://doi.org/10.1002/prot.25661

Utama, G. L., Suraloka, M. P., Rialita, T., & Balia, R. L. (2021). Antifungal and aflatoxin-reducing activity of β-glucan isolated from *Pichia norvegensis* grown on tofu wastewater. *Foods, 10*(11), 2619. https://doi.org/10.3390/foods10112619

Vila-Donat, P., Marín, S., Sanchis, V., & Ramos, A. J. (2018). A review of the mycotoxin adsorbing agents, with an emphasis on their multi-binding capacity, for animal feed decontamination. *Food and Chemical Toxicology, 114*, 246–259. https://doi.org/10.1016/j.fct.2018.02.044

Wang, X., Bai, Y., Huang, H., Tu, T., Wang, Y., Wang, Y., Luo, H., Yao, B., & Su, X. (2019). Degradation of aflatoxin B1 and zearalenone by bacterial and fungal laccases in presence of structurally defined chemicals and complex natural mediators. *Toxins, 11*(10), 609. https://doi.org/10.3390/toxins11100609

Wang, Y., Zhao, C., Zhang, D., Zhao, M., Zheng, D., Lyu, Y., … Cui, Z. (2017). Effective degradation of aflatoxin B1 using a novel thermophilic microbial consortium TADC7. *Bioresource Technology, 224*, 166–173. https://doi.org/10.1016/j.biortech.2016.11.033

Watanakij, N., Visessanguan, W., & Petchkongkaew, A. (2020). Aflatoxin B1-degrading activity from *Bacillus subtilis* BCC 42005 isolated from fermented cereal products. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment, 37*(9), 1579–1589. https://doi.org/10.1080/19440049.2020.1778182

Woo, C. S. J., & El-Nezami, H. (2015). Mycotoxins in Asia: Is China in danger? *Quality Assurance and Safety of Crops & Foods, 7*(1), 3–25. https://doi.org/10.3920/QAS2014.x005

Xia, X., Zhang, Y., Li, M., Garba, B., Zhang, Q., Wang, Y., Zhang, H., & Li, P. (2017). Isolation and characterization of a *Bacillus subtilis* strain with aflatoxin B1 biodegradation capability. *Food Control, 75*, 92–98. https://doi.org/10.1016/j.foodcont.2016.12.036

Xia, Y., He, R., Sun, Y., Zhou, H., Gao, M., Hu, X., Cui, X., Cheng, Q., & Wang, Z. (2022). Food-grade expression of manganese peroxidases in recombinant *Kluyveromyces lactis* and degradation of aflatoxin B1 using fermentation supernatants. *Frontiers in Microbiology, 12*, Article 821230. https://doi.org/10.3389/fmicb.2021.821230

Xing, F., Wang, L., Liu, X., Selvaraj, J. N., Wang, Y., Zhao, Y., & Liu, Y. (2017). Aflatoxin B1 inhibition in *aspergillus flavus* by *aspergillus Niger* through down-regulating expression of major biosynthetic genes and AFB1 degradation by atoxigenic *a. flavus*. *International Journal of Food Microbiology, 256*, 1–10. https://doi.org/10.1016/j.ijfoodmicro.2017.05.013

Xiong, J., Wen, D., Zhou, H., Chen, R., Wang, H., Wang, C., … Wu, L. (2022). Occurrence of aflatoxin M1 in yogurt and milk in Central-Eastern China and the risk of exposure in milk consumers. *Food Control, 137*, Article 108928. https://doi.org/10.1016/j.foodcont.2022.108928

Xu, L., Eisa Ahmed, M. F., Sangare, L., Zhao, Y., Selvaraj, J. N., Xing, F., … Liu, Y. (2017). Novel aflatoxin-degrading enzyme from *Bacillus shackletonii* L7. *Toxins, 9*(1), 36. https://doi.org/10.3390/toxins9010036

Xu, Y., Dong, H., Liu, C., Lou, H., & Zhao, R. (2023). Efficient aflatoxin B1 degradation by a novel isolate, *Pseudomonas aeruginosa* M-4. *Food Control, 149*, Article 109679. https://doi.org/10.1016/j.foodcont.2023.109679

Yang, P., Lu, S., Xiao, W., Zheng, Z., Jiang, S., Jiang, S., Jiang, S., Cheng, J., & Zhang, D. (2021). Activity enhancement of *Trametes versicolor* aflatoxin B1-degrading enzyme (TV-AFB1D) by molecular docking and site-directed mutagenesis techniques. *Food and Bioproducts Processing, 129*, 168–175. https://doi.org/10.1016/j.fbp.2021.08.007

Yang, P., Xiao, W., Lu, S., Jiang, S., Zheng, Z., Zhang, D., Zhang, M., Jiang, S., & Jiang, S. (2021). Recombinant expression of *Trametes versicolor* aflatoxin B1-degrading enzyme (TV-AFB1D) in engineering *Pichia pastoris* GS115 and application in AFB1 degradation in AFB1-contaminated peanuts. *Toxins, 13*(5), 349. https://doi.org/10.3390/toxins13050349

Yang, X., Zhao, Z., Tan, Y., Chen, B., Zhou, C., & Wu, A. (2020). Risk profiling of exposures to multiclass contaminants through cereals and cereal-based products consumption: A case study for the inhabitants in Shanghai, China. *Food Control, 109*, Article 106964. https://doi.org/10.1016/j.foodcont.2019.106964

Yehia, R. S. (2014). Aflatoxin detoxification by manganese peroxidase purified from *Pleurotus ostreatus*. *Brazilian Journal of Microbiology, 45*(1), 127–133. https://doi.org/10.1590/s1517-83822014005000026

Yu, J. (2012). Current understanding on aflatoxin biosynthesis and future perspective in reducing aflatoxin contamination. *Toxins, 4*, 1024–1057. https://doi.org/10.3390/toxins4111024

Yu, J., Bhatnagar, D., & Cleveland, T. E. (2004). Completed sequence of aflatoxin pathway gene cluster in *aspergillus parasiticus*. *FEBS Letters, 564*(1), 126–130. https://doi.org/10.1016/S0014-5793(04)00327-8

Zhang, D., Tian, Y., Tian, Y., Xing, H., Liu, S., Zhang, H., Ding, S., Cai, P., Sun, D., Zhang, T., Hong, Y., Dai, H., Tu, W., Chen, J., Wu, A., & Hu, Q.-N. (2021). A data-driven integrative platform for computational prediction of toxin biotransformation with a case study. *Journal of Hazardous Materials, 408*, Article 124810. https://doi.org/10.1016/j.jhazmat.2020.124810

Zhang, D., Xing, H., Liu, D., Han, M., Cai, P., Lin, H., … Hu, Q.-N. (2024). Discovery of toxin-degrading enzymes with positive unlabeled deep learning. *ACS Catalysis, 14*(5), 3336–3348. https://doi.org/10.1021/acscatal.3c04461

Zhang, H., Cui, L., Xie, Y., Li, X., Zhao, R., Yang, Y., … Jia, H. (2024). Characterization, mechanism, and application of dipeptidyl peptidase III: An aflatoxin B1-degrading enzyme from *aspergillus terreus* HNGD-TM15. *Journal of Agricultural and Food Chemistry, 72*(28), 15998–16009. https://doi.org/10.1021/acs.jafc.4c03531

Zhang, J., Chen, R., Zhou, H., Wen, D., Lu, Q., Xiong, J., & Wang, C. (2024). Prevalence of aflatoxin B1 in four kinds of fermented soybean-related products used as traditional Chinese food. *LWT, 191*, Article 115611. https://doi.org/10.1016/j.lwt.2023.115611

Zhang, L., Xu, W., Yue, P., Wang, Q., Li, Y., Pei, X., & Zeng, P. (2020). High occurrence of aflatoxin B1 in Pixian Doubanjiang, a typical condiment in Chinese cuisine. *Food Control, 110*, Article 107034. https://doi.org/10.1016/j.foodcont.2019.107034

Zhao, L. H., Guan, S., Gao, X., Ma, Q. G., Lei, Y. P., Bai, X. M., & Ji, C. (2011). Preparation, purification and characteristics of an aflatoxin degradation enzyme from *Myxococcus fulvus* ANSM068. *Journal of Applied Microbiology, 110*(1), 147–155. https://doi.org/10.1111/j.1365-2672.2010.04867.x

Zhou, L., Duan, X., Dai, J., Ma, Y., Yang, Q., & Hou, X. (2024). A covalent-organic framework-based platform for simultaneous smartphone detection and degradation of aflatoxin B1. *Talanta, 278*, Article 126505. https://doi.org/10.1016/j.talanta.2024.126505

Zhou, Z., Li, R., Ng, T. B., Huang, F., & Ye, X. (2022). Considerations regarding affinity determinants for aflatoxin B1 in binding cavity of fungal laccase based on *in silico* mutational and *in vitro* verification studies. *Ecotoxicology and Environmental Safety, 234*, Article 113412. https://doi.org/10.1016/j.ecoenv.2022.113412