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Microbial degradation of aflatoxin B1: Current status and future advances

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A B S T R A C T

Aflatoxin B1 (AFB1) is a natural toxin produced by many food-contaminant fungi and is a threat to human and animal health. This review summarizes current knowledge of the different ways to limit AFB1 in the food chain. We start by introducing current data and reviews available on the prevention of AFB1 occurrence, on AFB1 non-biological decontamination and biological adsorption. We then focus on microbial AFB1-degradation. The latter has already been well studied using living organisms, supernatants or purified enzymes. This review compiles information on the variety of protocols and the efficacy of the different sub-kingdoms or classes of microorganisms or their enzymes. We pay particular attention to publications closest to in vivo applications of microbial AFB1-degradation. In addition, this review also provides a summary of the currently known microbial degradation metabolites of AFB1 and their levels of toxicity, and provides recommendations on the most promising techniques to pursue the aim of minimizing AFB1 in the food supply.

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Abbreviations: AFB1, aflatoxin B1; AFB2, aflatoxin B2; AFB2a, aflatoxin B2a; AFD1, aflatoxin D1; AFG1, aflatoxin G1; AFG2, aflatoxin G2; AFO, aflatoxin oxidase; AFT, AFB1 + AFB2 + AFG1 + AFG2; ESI, electrospray ionisation; FDR, F₄₂₀H₂ dependent reductase; K_m, Michaelis constant; K_{cat}, catalysis constant; LCMS, liquid chromatography mass spectrometry; LAB, lactic acid bacteria; MnP, Mn peroxidase; QTOF, quadrupole time of fly.

1. Introduction

Certain species of *Aspergillus* produce toxic secondary metabolites, aflatoxins (AFT) being the most toxic and problematic. Of the aflatoxigenic *Aspergilli*, *A. flavus* produces only aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) whereas *A. parasiticus* produces also aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Those food contaminants are commonly found in cereals, nuts and spices (Azzoune et al., 2015; Masood et al., 2015; Riba et al., 2010). AFB1, AFB2, AFG1 and AFG2 are toxic for humans and animals. All the four aflatoxins are highly hepatotoxic, nephrotoxic, and immunotoxic. AFB1 is also carcinogenic for humans (IARC Publications list, 2012). To limit animal and human exposure, contamination by AFT is regulated worldwide (Wu and Guclu, 2012).

In the face of health risks and economic losses, scientists have sought solutions to limit AFT occurrence at pre and post-harvest stages. Thus, if we take the example of the maize food chain, prevention strategies can either act on the seeds (e.g.: Bt maize hybrids, resistance mechanisms), in the field (e.g.: good field practices, AFLA-maize, afla-guard®) (Dorner and Lamb, 2006) or during storage (e.g.: water activity (a_w) and temperature management) (Abbas et al., 2009; Warburton and Williams, 2014). Unfortunately, those various prevention techniques can be insufficient and AFT contamination still occurs. Risks may be more severe as AFB1 occurrence is expected to increase in Europe due to climate change (Battilani et al., 2016).

In terms of decontamination techniques, reviewed in Zhu et al., 2016, the most prevailing method for AFB1 decontamination is the addition of chemical adsorbents in feed. For example, the addition of bentonite (adsorbent) can prevent AFT adsorption in the gastrointestinal tract (European Union, 2013). Other numerous physical and chemical methods have been evaluated to reduce AFT content. Unfortunately, few conform with FAO requirements (Grenier et al., 2014): reduction of AFT without residual toxicity, guarantee of nutritional values and no modification of food or feed properties.

Therefore, researchers have focused on biological treatments for detoxification such as binding or degradation mechanisms. On one hand, biological binding seems promising but AFB1 may be easily released. On the other hand, biodegradation mechanisms are permanent. They modify the AFB1 structure, resulting in other molecules (e.g.: aflatoxicol) with potential unknown toxicity effects. Thus, further knowledge is needed on the identification, quantity and toxicity of degradation metabolites prior to the potential applications of biological treatments.

Throughout this review, we provide a critical assessment of the current research on how prevention and decontamination approaches can reduce AFB1 concentration. We then focus on the different classes and phyla of microorganisms involved in aflatoxin biodegradation. We also publish Tables detailing how efficiently bacteria, eukaryotes and their supernatants degrade AFB1. We identify the mechanisms of action, the examples of purified enzymes involved in the AFB1 degradation, their potential degradation products (chemical structures) and we discuss their associated toxicity. Finally, a proposed protocol is formulated for future degradation tests. This review concludes that those biodegradation agents are important, effective and eco-friendly solutions to decontaminate AFB1 in food and feed.

2. Reduction of aflatoxin occurrence

2.1. Prevention of aflatoxin occurrence

2.1.1. Planting

In maize, advances have been made in crop varieties. Two examples are the Genetic Enhancement of Maize program and the Maize Association Mapping Panel project (Henry et al., 2014; Warburton et al., 2013). These have identified a germplasm resistant to AFT accumulation. The lines they obtained are currently undergoing tests to produce suitable maize cultivars (Warburton and Williams, 2014). Other techniques

such as crop rotation, tillage to limit *Aspergillus* inoculum, early planting date are well known as key factors to minimize AFT occurrence (Munkvold, 2014).

2.1.2. Pre-harvest

Recent reviews have shown how farming methods can minimize AFT occurrence (Abbas et al., 2009; Gnonlonfin et al., 2012; Guo et al., 2012; Munkvold, 2014; Torres et al., 2014; Yazdanpanah and Eslamizad, 2015). The key findings focus on cultivation techniques (drought prevention), insect prevention, prediction models (AFLA-maize (Battilani et al., 2013)) and biocontrol strategies. The latter have strongly improved lately with the commercialization of atoxigenic *Aspergillus* section *Flavi* strains that can dominate toxigenic *Aspergillus*. Afla-guard® (Mehl and Cotty, 2010) and Aflasafe® (Atehnkeng et al., 2008) are the first biocontrol systems with a rate of potential AFT reduction above 90% (Grace et al., 2015).

2.1.3. Post-harvest

Storage at low water activity (a_w) (<0.73) and low temperature (<15 °C) results in low AFT production (Giorni et al., 2007). Sorting and removing moldy grains (reduced size) is a complementary approach that has recently been improved. New sorting techniques based on both optical (UV, infrared) and physical (size, density) parameters are currently under development (Womack et al., 2014).

2.2. Curative techniques

2.2.1. Adsorption

Many publications have highlighted how mineral adsorbents, especially clay minerals, effectively remove AFT (Deng et al., 2014; Di Gregorio et al., 2014; Vekiru et al., 2015). As an example, the first-ever adsorbent authorized by the EU is Mycofix® (Biomim, Herzogenburg, Austria). This bentonite-based adsorbent has a potential of AFT reduction above 90% (European Union, 2013).

For microbiological adsorbents, many research studies are ongoing, especially on lactic acid bacteria (LAB) (Bovo et al., 2015; Dogi et al., 2011; Fernández Juri et al., 2015; Hernandez-Mendoza et al., 2009; Joannis-Cassan et al., 2011; Pizzolitto et al., 2011; Rahaie et al., 2012; Topcu et al., 2010). However, this kind of adsorption mechanism is reversible in nature and has never been commercialized.

A promising approach to overcome those shortcomings could be a combination of both mineral and biological adsorbents to enhance effectiveness (Poloni et al., 2015).

2.2.2. Physical and chemical reductions

Other physical and chemical processes can partially reduce AFT content (Womack et al., 2014), especially in the maize food chain (Grenier et al., 2014). Those processes include extrusion, ammoniation, ozonation, nixtamalization, etc. Among them, ozonation, photodegradation and nixtamalization remain the most promising techniques (Chen et al., 2014; Grenier et al., 2014; Liu et al., 2012; Luo et al., 2013, 2014). Unfortunately, they do not comply with safety, cost and productivity requirements for commercialization.

2.2.2.1. Degradation. The degradation of AFB1 by plant extracts and microorganisms is extensively studied (Adebo et al., 2015; Iram et al., 2015, 2016). Those processes lead to degradation products of AFB1 that are expected to be less toxic than the parent molecule for humans and animals. This review aims to compile data available on microbial AFB1 degradation and potential detoxification, with a special focus on the results closest to industrial application.

Table 1

Cultures of bacteria tested in various conditions for their AFB1 reduction efficacy.

Class	Name	Medium	Inoculation condition	Inoculum (CFU/ml)	[AFB1] (mg/kg)	Temperature (°C)	Incubation period	Reduction efficacy	References	
Actinobacteria	Actinobacteria (12)	ISP-2	On Petri dishes	NS	5	28	4 d	0–84.4%	Verheecke et al. (2014)	
	<i>Brachybacterium</i> sp.	NB	In liquid medium	NS	0.1	37	72 h	74.8%	Guan et al. (2008)	
	<i>Cellulosimicrobium</i> sp.	NB	In liquid medium	NS	0.1	37	72 h	74.8%	Guan et al. (2008)	
	<i>Corynebacterium rubrum</i>	n°3 medium	In liquid medium	5.10 ⁷	1.48	28	4 d	99.1%	Mann and Rehm (1977)	
	<i>Mycobacterium smegmatis</i>	PYB	In liquid medium	NS	6	28	48 h	~100%	Taylor et al. (2010)	
	<i>Nocardia corynebacterioides</i>	Czapek-Dox medium	On Petri dishes	NS	7.5	28	44 h	74.0%	Ciegler et al. (1966)	
	<i>N. corynebacterioides</i>	Chick feed (corn-soybean)	On feed	2.10 ⁷⁻⁸	2.3 ^a	28	72 h	24.7%	Tejada-Castañeda et al. (2008)	
	<i>N. asteroides IFM 8</i>	Water bath	bacteria	NS	12	37	38 h	~100%	Arai et al. (1967)	
	<i>Rhodococcus erythropolis DSM 14303</i>	Standard I broth	In liquid medium	NS	1.75	30	72 h	93–97%	Teniola et al. (2005)	
	<i>R. erythropolis</i> (6)	LB medium	In liquid medium	OD ₆₀₀ : 0.6	2	28	72 h	87.14–99.96%	Krifaton et al. (2011)	
	<i>R. strains: - R. erythropolis</i> (15)	LB medium	In liquid medium	OD ₆₀₀ : 0.6	2	28	72 h	Up to 100%	Cserhádi et al. (2013)	
	<i>R. sp.</i>	NB	In liquid medium	NS	0.1	37	72 h	73.9%	Guan et al. (2008)	
	<i>R. erythropolis</i> ATCC 4277	Difco ISP No.1	In liquid medium	NS	20	30	24 h	95.9%	Eshelli et al. (2015)	
	- <i>R. globerulus</i> AK36	LB medium	In liquid medium	OD ₆₀₀ : 0.6	2	28	72 h	95.0%	Cserhádi et al. (2013)	
	- <i>R. pyridinivorans</i> (4) and <i>R. rhodochrous</i> (2)	LB medium	In liquid medium	OD ₆₀₀ : 0.6	2	28	72 h	98.0%	Cserhádi et al. (2013)	
	<i>R. rhodochrous</i> NI2 & ATCC 12674	LB medium	In liquid medium	OD ₆₀₀ : 0.6	2	28	72 h	96.1–99.98%	Krifaton et al. (2011)	
	<i>R. pyridinivorans</i>	LB medium	In liquid medium	OD ₆₀₀ : 0.6	2	28	72 h	82.1–99.9%	Krifaton et al. (2011)	
	<i>Streptomyces lividans</i> TK24	Difco ISP No. 1	In liquid medium	NS	20	30	24 h	86.1%	Eshelli et al. (2015)	
	<i>Str. aureofaciens</i> ATCC 10762	Difco ISP No. 1	In liquid medium	NS	20	30	24 h	88.0%	Eshelli et al. (2015)	
	<i>Str. cacaoi</i> subsp. <i>asoensis</i>	LB medium	Supernatant	OD ₆₀₀ : 0.6	1.136	28	5 d	88.4%	Harkai et al. (2016)	
	Bacillus	<i>Bacillus</i> TUBF1	MSM	In liquid medium	0.65	10	30	72 h	100.0%	El-Deeb et al. (2013)
		<i>B. spp.</i> (23)	NB	In liquid medium	NS	5	37	7 d	0–69%	Petchkongkaew et al. (2007)
		<i>B. sp.</i> (2)	NB	In liquid medium	NS	0.1	37	72 h	80.9–77.8%	Guan et al. (2008)
<i>B. licheniformis</i> CFR1		NB	In liquid medium	NS	0.5	37	72 h	94.7%	Raksha Rao et al. (2016)	
<i>B. subtilis</i> UTBSP1		Pistachios nuts	Bacteria	NS	0.002	30	5 d	95.0%	Farzaneh et al. (2012)	
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> and <i>Streptococcus thermophilus</i>		Pre-heated peanut meal	Anaerobic solid fermentation	3.10 ³ /7.10 ⁸	0.0105 + 0.0187 (AFG1)	37	3 d	100.0%	Chen et al. (2015)	
<i>L. plantarum</i> (PTCC 1058)		Corn samples	On corn	9.10 ⁹	0.24	37	4–7 d	77.0%	Khanafari et al. (2007)	
<i>Lysinibacillus fusiformis</i>		NB	In liquid medium	NS	0.005	37	48 h	61.3%	Adebo et al. (2016b)	
<i>Sporosarcina</i> sp.		NB	In liquid medium	NS	0.005	37	48 h	46.9%	Adebo et al. (2016b)	
<i>Staphylococcus</i> sp. VGF2		NB	In liquid medium	NS	0.005	37	48 h	56.8%	Adebo et al. (2016a)	
<i>Staphylococcus warneri</i>		NB	In liquid medium	NS	0.005	37	48 h	47.4%	Adebo et al. (2016b)	
<i>Streptococcus lactis</i> (ATCC-11,454)		Sterilized skim milk	In liquid medium	0.5% of active starter	0.22	30	15 d	NS (non quantitative)	Megalla and Mohran (1984)	
γ-Proteobacteria		<i>Enterobacter</i> sp.	NB	In liquid medium	NS	0.1	37	72 h	75.9%	Guan et al. (2008)
		<i>Klebsiella</i> sp.	NB	In liquid medium	NS	0.1	37	72 h	77.5%	Guan et al. (2008)
	<i>Pseudomonas aeruginosa</i> N17–1	NB	In liquid medium	NS	0.1	37	72 h	82.8%	Sangare et al. (2014)	
	<i>Pseudomonas anguilliseptica</i> VGF1	NB	In liquid medium	NS	0.005	37	48 h	51.7%	Adebo et al. (2016a)	

(continued on next page)

Table 1 (continued)

Class	Name	Medium	Inoculation condition	Inoculum (CFU/ml)	[AFB1] (mg/kg)	Temperature (°C)	Incubation period	Reduction efficacy	References
	<i>Pseudomonas fluorescens</i>	NB	In liquid medium	NS	0.005	37	48 h	47.7%	Adebo et al. (2016a)
	<i>P. putida</i> MTCC 1274 and 2445	MSG	Bacterial pellets	9. 10 ⁵	0.2	37	24 h	90.0%	Samuel et al. (2014)
	<i>Stenotrophomonas</i> sp. (10) including <i>S. sp.</i> NMO-3	BM	In liquid medium	NS	0.1	37	72 h	85.7%	Liang et al. (2008)
	<i>S. maltophilia</i> 35–3	NB	In liquid medium	NS	0.1	37	72 h	82.5%	Guan et al. (2008)
α-p	<i>Brevundimonas</i> sp. (2)	NB	In liquid medium	NS	0.1	37	72 h	78.1–76.8%	Guan et al. (2008)

α-p.: α-proteobacteria; BM: broth medium (Aoboxing Co., Beijing); ISP-2: (Shirling and Gottlieb, 1966); MSG: mineral salt glucose medium; NB: nutrient broth; ND: not detected; NS: not studied; PYB: peptone yeast extract broth (9 g/l peptone, 4.5 g/l yeast extract, 23 mM Na₂HPO₄, 88 mM KH₂PO₄, 9 mM NaCl, pH 6.0); standard I broth: composition available in Goodfellow, Str.: *Streptomyces*; 1986.

^a Cocktail of AFB1 + B2 + G1 + G2 + aflatoxicol tested.

3. Focus on microbial degradation

3.1. Living microorganisms

3.1.1. Bacteria

The removal of AFB1 by microbial degradation has been investigated since the late 1960s. The first bacterium identified as an AFB1 degrader was *Nocardia corynebacterioides* (previously known as *Flavobacterium aurantiacum*). On Czapek-Dox medium a 74% AFB1 degradation was demonstrated after 44 h at 28 °C (Ciegler et al., 1966). Since then, many studies using bacteria have been carried out. Table 1 summarizes those studies according to the bacterial classes tested. Many studies have tested the impacts of living bacteria belonging to the Actinobacteria (Gram positive), *Bacillus* (Gram positive) and α or β-proteobacteria (Gram negative) classes on AFB1 degradation (Table 1).

The Actinobacteria class was widely tested, mainly in liquid medium. Its AFB1 degradation capacity varied between 24 and 100%. Only one publication showed results on solid medium and found that *Streptomyces* strains degraded AFB1 (5 mg/kg) by 84.4% after 4 days at 28 °C (Verheecke et al., 2014). α and β-proteobacteria classes were tested in liquid media. Their degradation efficacy ranged from 47.7 to 90%. The best AFB1 degradation capacity was achieved with bacterial pellets of *Pseudomonas putida* MTCC 1274 and MTCC 2445 after 24 h at 37 °C in the presence of 0.2 mg/l of AFB1.

The compilation of data represented in Table 1 shows no correlation between class affiliation and reduction efficacy. Despite the heterogeneity of test protocols (differences in inoculum conditions, medium, AFB1 concentration, temperature, incubation period, etc), effectiveness in AFB1 degradation seemed to be strain specific. Indeed, El-Deeb et al. (2013) identified the strain *Bacillus* TUBF1 as able to remove 100% AFB1r in MSM liquid medium after a 3 days incubation at 30 °C. However, Petchkongkaew et al. (2007) tested 23 *Bacillus* spp. strains and obtained between 0 and 69% AFB1 removal after an incubation of 7 days at 37 °C in NB liquid medium. Those examples highlight how the efficacy of AFB1 degradation within the same genus may vary widely.

In vitro AFB1 microbial degradation is usually difficult to transfer to food matrices.

However, recent tests have revealed promising results. The following examples are presented in increasing order of efficacy. Tejada-Castañeda et al. (2008) studied the potential of *N. corynebacterioides* as an AFB1 degrader. First, chick feed (soybean based) that was contaminated with *A. flavus* then maintained for 2 weeks at 24 °C reached a concentration of 2.3 mg/kg AFT (+ aflatoxicol B). The autoclaved feed was then incubated for 3 days at 28 °C with *N. corynebacterioides* and a degradation of 25% was observed for AFT (+ aflatoxicol B), including 32% AFB1 degradation.

LAB can achieve higher efficacies in food matrices. For example, Khanafari et al. (2007) tested *Lactobacillus plantarum* PTCC 1058 on

corn samples artificially contaminated with 0.24 mg/kg of pure AFB1. The efficacy of AFB1 degradation was 77% after 7 days at 37 °C.

Two AFB1 degrading bacterial strains recently achieved the best efficiencies. Firstly, Farzaneh et al. (2012) demonstrated 95% AFB1 degradation by *Bacillus subtilis* UTBSP1 on shell nuts. Secondly, Chen et al. (2015) showed 100% AFB1 removal by the mixed treatment of *L. delbrueckii* subsp. *Bulgarius* and *Streptococcus thermophilus* on pistachio nuts. However, caution is needed as those two studies used an initial AFB1 concentration much lower (in ppb) than the other in vitro tests previously described and presented in Table 1.

3.1.2. Eukaryotes

The removal of AFB1 by eukaryotes has also been studied since the late 1960s. Ciegler et al. (1966) tested various yeasts, fungi and algae for ability to degrade AFB1, and some *Aspergillus* and *Penicillium* strains (on Czapek-Dox medium) showed positive results. Since 1966, many studies have been conducted to test AFB1 degradation by numerous eukaryotes. Table 2 summarizes the AFB1 degradation by eukaryotes depending on the sub-kingdoms tested: Ascomycota, Basidiomycota, Zygomycota and *Tetrahymena pyriformis*, representing the Chromalveolata Kingdom.

T. pyriformis was incubated with 2.4 mg/l of AFB1 and showed 67% AFB1 degradation after 48 h at 25 °C on NB liquid medium. Unfortunately, *T. pyriformis* did not degrade AFG1 at the same level (Teunisson and Robertson, 1967). However, as this fresh water ciliate is often used as a model for ecotoxicity studies (Sauvant et al., 1999), it could be interesting to further research its degradation potential.

The ability of Ascomycota and Zygomycota to degrade AFB1 varies between 50% and 100% (Table 2). The highest AFB1 degradation was obtained recently by Hackbart et al. (2014) who found *Trichoderma reesii* QM9414 (Ascomycota) and *Rhizopus oryzae* CCT7560 (Zygomycota) could degrade 100% AFB1 in PDA medium after 5 days at 30 °C. However, no tests of Ascomycota and Zygomycota were undertaken on food matrices.

For Basidiomycota, the potential of *Pleurotus ostreatus* to degrade AFB1 has been studied for > 15 years. Recently, Das et al. (2014) tested 2 strains of *P. ostreatus* on autoclaved rice. AFB1 was degraded between 89 and 92% after an incubation of 15 days at 30 °C. Those first promising results have to be confirmed on naturally contaminated food matrices prior to potential application.

Another approach is to use species of *Aspergillus* (Ascomycota) as the genus is known to contain AFB1 degraders (Doyle et al., 1982; Hamid and Smith, 1987; Huynh and Lloyd, 1984). Within this genus, some species and strains produce aflatoxins while others do not. The atoxigenic species could also be potential AFB1 degraders (Hamid and Smith, 1987). The main example is *A. niger* which can degrade 58% of AFB1 after an incubation of 2 days at 32 °C (Table 2).

Table 2

Cultures of eukaryotes tested in various conditions for their efficacy to degrade AFB1.

Kingdom	Name	Medium	Condition	Inoculum (CFU/ml)	[AFB1] (mg/kg)	Temperature (°C)	Incubation period	Reduction efficiency	References
Ascomycota	<i>Alternaria</i> sp.	Czapek Dox-casamino acid	In liquid medium	NS	0.5	28	5 d	83%	Shantha (1999)
	<i>As. candidus</i> Link	Czapek modified	In liquid medium	2.10 ⁵	50	25	10 d	88.50%	Lafont and Lafont (1974)
	<i>As. niger</i> ND-1	MTM + tryptone 0.5% + starch 4%	In liquid medium	NS	0.01	32	48 h	58.20%	Zhang et al. (2014)
	<i>As. niger</i>	Modified Czapek-Dox	On Petri dishes	NS	0.25	Room	After 11 days	NQ reduction	Ciegler et al. (1966)
	<i>Dactylium dendroides</i>	Yes	In liquid medium	NS	4	30	24–48 h	50–60%	Detroy and Hesselstine (1969)
	<i>Neurospora</i> 429	Groundnut extract	On Petri dishes	1.10 ⁵	0.89	30	Up to 3 weeks	84.30%	Nout (1989)
	<i>Penicillium raistrickii</i> NRRL 2038	Modified Czapek-Dox	On Petri dishes	NS	0.25	Room temperature	Up to 2 weeks	NQ reduction	Ciegler et al. (1966)
	<i>Phoma</i> sp.	Czapek-Dox-casamino acid	In liquid medium	NS	0.5	28	4 d	99%	Shantha (1999)
	<i>Trichoderma</i> sp. 639	Czapek-Dox-casamino acid	In liquid medium	NS	0.5	28	5 d	83%	Shantha (1999)
	<i>Tr. Reesi</i> QM9414	PDA	On Petri dishes	4.10 ²	0.1	30	5 d	100%	Hackbart et al. (2014)
Basidiomycota	<i>Pleurotus ostreatus</i> GHBBF10	Rice	By plugs	NS	0.5	30	15 d	89%	Das et al. (2014)
	<i>Pl. ostreatus</i> MTCC 142	Rice	By plugs	NS	0.5	30	15 d	92%	Das et al. (2014)
	<i>Sporotrichum</i> (2)	Czapek-Dox-casamino acid	In liquid medium	NS	0.5	28	5 d	83%	Shantha (1999)
Zygomycota	<i>Absidia repens</i>	Yes	Liquid medium	NS	4	30	24–48 h	50–60%	Detroy and Hesselstine (1969)
	<i>Mucor griseo-cyanus</i>	Yes	Liquid medium	NS	4	30	24–48 h	50–60%	Detroy and Hesselstine (1969)
	<i>Rhizopus arrhizus</i> , <i>R. arrhizus</i> NRRL 2582, <i>R. oryzae</i> NRRL 395, <i>R. stolonifer</i> NRRL 1477	WP	In liquid medium	NS	117.6	27	7 d	58%	Cole et al. (1972)
	<i>Rhizopus</i> 581	Groundnut extract	On Petri dishes	1.10 ⁵	0.89	30	Up to 3 weeks	67%	Nout (1989)
	<i>Rhizopus</i> spp. (3)	Czapek-Dox-casamino acid	In liquid medium	NS	0.5	28	5 d	0–83%	Shantha (1999)
	<i>R. oryzae</i> CCT7560	PDA	On Petri dishes	4.10 ²	0.1	30	5 d	100%	Hackbart et al. (2014)
Chromalveolata	<i>Tetrahymena pyriformis</i> W	NB	In liquid medium	2.2 10 ⁷	2.4	25	48 h	67%	Teunisson and Robertson (1967)

MTM: 5.0 g peptone, 1.0 g K₂HPO₄, 2.0 g yeast extract, 0.5 g MgSO₄ and 20.0 g glucose; modified Czapek-Dox medium: sucrose, 3.0%; NaNO₃, 0.3%; K₂HPO₄, 0.1%; MgSO₄, 0.05%; Cl, 0.05%; FeSO₄, 0.001%; yeast extract (Difco), 0.005%; agar, 2.0%; nkat: nmol/l/s; NQ: non quantitative, NB: nutrient broth, PDA: potatoes dextrose agar.

3.2. Mechanisms

Different mechanisms are involved in AFB1 degradation by Bacteria and Eukaryota. This review shows how degradation occurs mostly through the action of extracellular enzyme(s). Such enzymes could be valuable assets for the food industry.

3.2.1. Microbial culture supernatants

In most cases, the culture supernatant is identified as the degrading matrix, thus microbial culture supernatants have been widely studied for their AFB1 degradation potential. Table S1 lists culture supernatants and their ability to degrade AFB1. Culture supernatants of Actinobacteria, Bacillus and γ -proteobacteria classes were tested. *Mycobacterium fluoranthenorans* DSM 44556T (Actinobacteria) had the highest effectiveness. Its supernatant was able to reduce 100% of AFB1 (initial concentration 2.5 mg/l) after 24 h at 30 °C. However, only few studies analyzed the residual toxicity of the degradation products. For example, the culture supernatants of *Bacillus TUBF1*, *Cellulosimicrobium funkei* and *Rhodococcus erythropolis* DSM 14303, degraded AFB1 by 67 to

90% and their unidentified degradation products were less toxic than AFB1 (Teniola et al., 2005; El-Deeb et al., 2013; Sun et al., 2015).

For all the examples presented in Table S1, the nature of the enzymatic activities which degrade AFB1 has not yet been elucidated. As an example, Alberts et al. (2009) studied the AFB1-degradation potential of the culture filtrates *Aspergillus niger* D15-Lcc2#3, *Peniophora* sp. SCC0152 and *Pleurotus ostreatus* St2–3. AFB1 degradation by these filtrates were respectively 55%, 52.4% and 76% (from 1.4 mg/kg) after 3 days at 30 °C. The authors concluded that there could be a causal link between the extract's laccase activity and AFB1 degradation.

Finally, the great variability of the reaction parameters tested (time, buffer, concentration and toxicity tests) makes it difficult to compare AFB1 degradation among the examples cited in this review. In addition, further research is needed prior to their application in food matrices.

3.2.2. Enzymes

All the enzymes currently known for their AFB1 degradation ability are summarized in Table 3. Among the different supernatants tested, only a few studies identified the enzyme(s) involved. In 1998, aflatoxin

Table 3
Purified enzymes identified as AFB1 degraders.

Enzyme	Family	Efficacy	Conditions	Structure ^a	Producing organism	Optimal conditions	References
FDR-A FDR-B	F ₄₂₀ H ₂ dependent reductase	100%	30 µM AFB1; 10 µM F ₄₂₀ up to 24 h	YP_887663.1 & YP_887686.1	<i>Mycobacterium smegmatis</i>	NS	Taylor et al. (2010)
MADE	NS	96.96% (AFG1)	100 ng/ml AFG1; 80 U/ml MADE	NS	<i>Myxococcus fulvus</i> ANSM068	pH 6, 35 °C, Mg ²⁺ , inhibited by Zn ²⁺	Zhao et al. (2011)
ADTZ/AFO	Oxidase	NQ reduction	0.2 mg/l AFB1; 0.1 mg/ml AFO	AAX53114.1	<i>Armillariella tabescens</i>	pH 6, 35 °C, Mg ²⁺ , inhibited by Zn ²⁺	Cao et al. (2011), Liu et al. (2001), Wu et al. (2015), and Yao et al. (1998)
MnP	Mn peroxidase	86%	50 mg/l AFB1 AFB1; 5nkat	NS	<i>Phanerochaete sordida</i> YK-624	NS	Wang et al. (2011)
90 kDa protein	NS	NQ reduction	5 mg/l AFB1; 50 mg/ml 90 kDa protein	NS	<i>Pleurotus ostreatus</i>	NS	Motomura et al. (2003)
42 kDa protein	Mn peroxidase	90%	312 mg/l AFB1; 1.5 U/ml	BAL61124.1	<i>Pleurotus ostreatus</i>	25 °C, pH 4–5, Mn ²⁺ , Cu ²⁺ , Ca ²⁺ , K ⁺ inhibited by Hg ²⁺ , Cd ²⁺	Yehia (2014)
Laccase	Laccase	89%	1.4 mg/l AFB1; 1 U/ml laccase	NS	<i>Trametes versicolor</i>	NS	Alberts et al. (2009)

ADTZ/AFO: aflatoxin oxidase; MADE: Myxobacteria aflatoxin degradation enzyme; Mn: manganese; MnP: manganese peroxidase; NQ: non quantitative.

^a If possible, NCBI accession number.

oxidase (AFO) was the first enzyme identified as able to degrade AFB1 (Yao et al., 1998). AFO is the only AFB1 degrading enzyme isolated from intracellular extracts. Using Michaelis constant (K_m), recent publications have highlighted how AFO has a strong affinity with AFB1 ($K_m = 0.334 \mu\text{M}$) and its intermediate sterigmatocystin (ST) ($K_m = 0.106 \mu\text{M}$). However, the catalysis constant (K_{cat}) of AFO with AFB1 was relatively low: 0.045 s^{-1} (Wu et al., 2015). The opposite was observed with the F₄₂₀H₂ dependent reductase (FDR) from *Mycobacterium smegmatis*. Indeed, the enzyme MSMEG_5998 (FDR-A protein) has a higher K_m (47 μM) and a higher K_{cat} (63 min^{-1}) (Taylor et al., 2010) than AFO. This enzyme thus has less affinity to AFB1 than AFO but has a higher catalysis activity.

Less data are available for the other purified enzymes. Wang et al. (2011) studied a Mn peroxidase (MnP) purified from *Phanerochaete sordida* YK-624. They found that $5.10 \cdot 10^{-9} \text{ mol/s}$ of MnP can catalyse the conversion of 86% of AFB1 (50 mg/l) in 48 h at 30 °C. Unfortunately, no data are available yet on the K_m and K_{cat} of this MnP as well as for the other enzymes represented in Table 3.

These enzymes may have different targets on the AFB1 molecule. AFO and MnP catalyse the bisfuran ring of AFB1 and FDR-A catalyses the α,β -moiety ester (unstable) of AFB1 (Taylor et al., 2010; Wang et al., 2011; Wu et al., 2015). The different active sites lead to a variety of AFB1 degradation products.

3.3. Degradation products

As a high AFB1 degradation potential is not always linked to the suppression of toxicity, it is crucial to study AFB1 degradation products and their respective toxicity. Among the latter, the only degradation product more toxic than AFB1 is AFB1-8,9-epoxide (carcinogenic form of AFB1), the result of the transformation in humans of AFB1 by cytochrome P450 (Vincenzi et al., 2011).

3.3.1. Identified degradation metabolites

In some rare cases, no degradation products were reported (Alberts et al., 2009; Raksha Rao et al. (2016); Sangare et al., 2014). For instance, when Sangare et al. (2014) studied the degradation capacity of *Pseudomonas aeruginosa* N17-1, as shown in Table S1, their LC-QTOF/MS analysis did not detect any residual metabolites from AFB1 degradation. Similar results were obtained after AFB1 degradation by the purified laccase from *Trametes versicolor* (Table 3): no degradation products were detected by ESIMS and LCMS (Alberts et al., 2009).

The first discovery of AFB1 degradation products came from Detroy and Hesseltine (1969). They identified a “new fluorescent-blue

compound” after incubation of *Absidia repens* with AFB1 (4 mg/kg). Since then, many metabolites from AFB1 degradation have been studied and are represented in Table 4. Aflatoxicol, aflatoxin B2a (AFB2a) and aflatoxin D1 (AFD1) were the most reported AFB1 degradation products. AFB1 degradation can be initiated at different cleavage points including the bisfuran ring (e.g.: AFB2a), the pentan group (e.g.: aflatoxicol) or the coumarin group (e.g. AFD1). The degradation mechanism from AFB1 to AFD1 was entirely elucidated recently (Eshell et al., 2015).

Adebo et al. (2015) reviewed the degradation mechanisms of the metabolites listed in Table 4. The authors highlighted three major insights. Firstly, they highlighted a variety of sites in the modification of AFB1 structure including aflatoxicol from living *Rhizopus* spp. or *A. flavus*, an opened bisfuran ring from *Armillariella tabescens* supernatant, etc. Secondly, they highlighted newly discovered degradation products (e.g.: C₁₇H₁₄O₇, C₁₆H₁₄O₅, etc) that need further characterization. Lastly, they also highlighted a lack of characterization of the degradation processes and their links to toxicity of degradation products.

3.3.2. Toxicity

Most of the metabolites identified were tested for their toxicity (except for the unstable reduced AFB1 and the 8,9-unsaturated carbon of AFB1) (Table 4). All these metabolites showed a reduced toxicity compared to AFB1 (e.g. AFB2A, AFD2) or a complete lack of toxicity (e.g. AFD1).

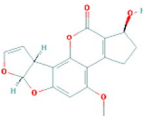
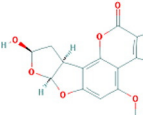
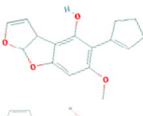
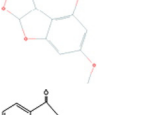

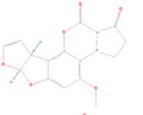
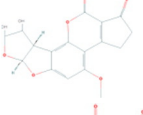
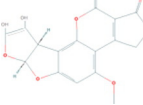
Another approach is to study the removal of toxicity within the treated condition. This approach was used by many authors as listed in the data in brief. Most of the tests used for detoxification assessment were in vitro: SOS-chromotest (mutagenicity assessment) and MTT test (cytotoxicity assessment). Nonetheless, some researchers went further and undertook in vivo testing using ducking and chick feeds (Tejada-Castañeda et al., 2008; Sun et al., 2015).

4. Conclusion

There are many approaches to reduce AFB1 occurrence in food and feed. If prevention techniques do not fully avoid AFB1 contamination, decontamination techniques such as chemical, physical or microbial treatments can help to remove a part of the remaining AFB1 content. Among them, microbial degradation is the most promising technique as it limits food and feed nutritional losses. This review has highlighted the diversity of protocols used and of microorganisms tested until now. Some were tested as whole cultures (listed in Tables 1 and 2) while others were tested as supernatants (listed in Table S1) or purified

Table 4

Representation of the known stable metabolites of aflatoxin B1 microbial degradation.

Metabolite	Structure	MW	Toxicity	Degrader origin	References
Aflatoxicol		314	18 times less toxic than AFB1, can form adduct to DNA	<i>Corynebacterium rubrum</i> ^a , <i>Nocardia corynebacterioides</i> , <i>Streptococcus lactis</i> (ATCC-11454), <i>Aspergillus niger</i> , <i>Eurotium herbariorum</i> , <i>Rhizopus</i> sp., <i>A. flavus</i>	Doyle et al. (1982), Karabulut et al. (2014), Mann and Rehm (1977), Megalla and Mohran (1984), and Nakazato et al. (1990)
Aflatoxin B2a		314	200 times less toxic than AFB1, relatively non toxic	<i>Lactobacillus delbrueckii subsp.bulgaricus</i> and <i>Streptococcus thermophilus</i> ^a , <i>Streptococcus lactis</i> (ATCC-11454), <i>Pleurotus ostreatus</i> GHBBF10, <i>Rhizopus arrhizus</i> , <i>R. arrhizus</i> NRRL 2582, <i>R. oryzae</i> NRRL 395, <i>R. stolonifer</i> NRRL 1477	Chen et al. (2015), Cole et al. (1972), Das et al. (2014), Megalla and Mohran (1984), and Wu et al. (2009)
Aflatoxin D1		286	Nontoxic towards Hela cells (2–6 µg·ml ⁻¹)	<i>Lactobacillus delbrueckii subsp.bulgaricus</i> and <i>Streptococcus thermophilus</i> ^a , <i>Pseudomonas putida</i> MTCC 1274 and 2445, <i>Rhodococcus erythropolis</i> ATCC 4277	Chen et al. (2015) and Eshelli et al. (2015)
Aflatoxin D2		206	Much less toxic than AFB1 towards Hela cells (EC ₅₀ 5.2 µg·ml ⁻¹)	<i>Pseudomonas putida</i> MTCC 1274 and 2445, <i>Rhodococcus erythropolis</i> ATCC 4277	Eshelli et al. (2015) and Samuel et al. (2014)
Phthalic anhydride		149	Much less toxic than AFB1 towards Hela cells (EC ₅₀ 7.5 µg·ml ⁻¹)	<i>Pseudomonas putida</i> MTCC 1274 and 2445	Samuel et al. (2014)
Reduced AFB1 (unstable)		310	NS	<i>Mycobacterium smegmatis</i>	Taylor et al. (2010)
8,9 unsaturated carbon of AFB1		346	NS	<i>Armillariella tabescens</i>	Cao et al. (2011), Liu et al. (2001), and Wu et al. (2015)
AFB1–8,9-dihydrodiol		344	Less mutagenic than AFB1 according to umu test on S9 liver homogenate	<i>Phanerochaete sordida</i> YK-624	Wang et al. (2011)

MW: molecular weight, NS: not studied.

^a Suspected.

enzymes for AFB1 degradation (listed in the data in brief). Those promising results were most often limited to in vitro conditions.

The results summarized in the review highlight the link between microbial degradation of AFB1 and reduction of toxicity in the treated samples. Nevertheless, degradation metabolites such as aflatoxicol (less toxic than AFB1) can still generate potential toxicity effects (Karabulut et al., 2014). The review of toxicity tests reveals a lack of in vivo tests and proposes the *C. funkei* test as the most advanced study towards potential feed application (Sun et al., 2015).

5. Insights for future AFB1 degradation tests

The microbial degradation of AFB1 is a promising field, to be developed in the near future. However, the heterogeneity of the tested conditions and the microorganisms studied has led to difficulties in comparing results. Hereafter, we suggest insights for future studies on AFB1 degraders.

Two selection criteria can be chosen for potential AFB1 degraders. One approach is the selection of microorganisms using coumarin as carbon source (Guan et al., 2008). The other approach is to randomly test known degraders of pollutants (Verheecke et al., 2014). The results compiled in this review highlight the diversity of AFB1 degradation sites, especially those linked to the bisfuran ring. Thus, when studying AFB1 degraders, attention should be paid not only to the coumarin degradation site but also to the potential food and feed application.

The second step is the protocol used to test AFB1 degradation. Taking into account that every microorganism has its own requirements, we

propose the use of recommended liquid medium at a 1 mg/l AFB1 concentration. The recommended media could be Czapek-dox for Eukaryota or nutrient broth for Bacteria (Table 1, Table 2). Throughout the review, we highlighted the wide concentration of AFB1 used for microbial degradation (2 µg/l to 117.5 mg/l). A standard concentration to compare the future tests could be 1 mg/l AFB1.

Moreover, protocols should include inoculum concentration and degradation tests on all AFT. Indeed, a lack of data remains on all the currently tested AFB1 degraders and their potency towards other AFT. The inoculation periods tested are very different but a pattern emerges of 3 days for bacteria (28–37 °C) and 7 days for fungi (25–30 °C).

After validating AFB1 degradation effectiveness, the next step is to characterize the extra or intracellular extracts. Despite the fact that many studies have focused on supernatants (Table S1), the AFO enzyme, of significant AFB1 degradation ability, comes from intracellular extracts (Liu et al., 2001). The analysis of intra/extracellular AFB1 degradation should be coupled with a LC-MS (Q-TOF or ESI) to identify potential degradation metabolites. This protocol will help to eliminate AFB1 degraders with aflatoxicol or even aflatoxin-8,9-epoxide as degradation metabolites. To the best of our knowledge, both metabolites are the most toxic degradation metabolites. Moreover, a toxicity test should be done in vitro (SOS-chromotest or MTT test) and in vivo (duckling or chick feed) to calculate the detoxification efficacy.

The candidates validated through all the previous steps should be analyzed in in vivo tests on feed. The protocol developed by Sun et al. (2015) can be taken as a reference. A slight improvement could be the use of contaminated feed for AFB1 ingestion.

By applying these recommendations, we could have comparable data between the different degradation solutions. The future of those methods is the development and formulation of cocktails of AFB1 degraders to optimise efficacy and provide solutions to the agro-food chain. The demand for this kind of biological agents will further increase in the future.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ijfoodmicro.2016.07.028>.

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