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Enzymatic transformation of aflatoxin B_1 by Rh_DypB peroxidase and characterization of the reaction products

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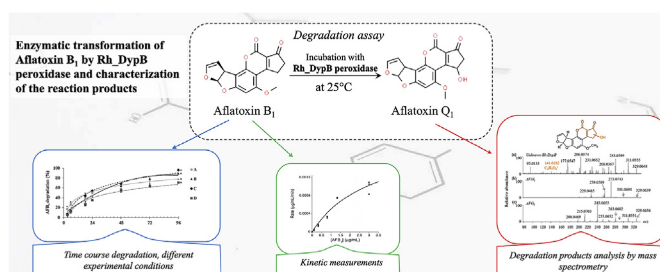
HIGHLIGHTS

- Rh_DypB application could be of great interest for the treatment of contaminated feed.
- Rh_Dyp B peroxidase was applied for aflatoxin B_1 biotransformation.
- Low enzyme and H_2O_2 concentrations were effective to reduce Aflatoxin B_1.
- Aflatoxin B_1 was quantitatively converted to Aflatoxin Q_1.
- AFQ_1 has a lower acute toxicity and mutagenicity than AFB_1.

GRAPHICAL ABSTRACT



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ABSTRACT

In some environments, a number of crops, notably maize and nuts can be contaminated by aflatoxin B_1 and related compounds resulting from the growth of aflatoxin-producing *Aspergilli*. Fungal peroxidases have been shown to degrade a number of mycotoxins, including aflatoxin B_1 (AFB_1). Therefore, the purpose of this study was to investigate the *in vitro* enzymatic degradation AFB_1 by a recombinant type B dye decolorizing peroxidase (Rh_DypB). Analysis of the reaction products by HPLC-MS analysis showed that under optimized conditions AFB_1 was efficiently transformed by Rh_DypB, reaching a maximum of 96% conversion after 4 days of reaction at 25 °C. Based on high resolution mass spectrometry analysis, AFB_1 was demonstrated to be quantitatively converted to AFQ_1, a compound with a significantly lower toxicity. A number of low molecular mass compounds were also present in the final reaction mixture in small quantities. The results presented in this study are promising for a possible application of the enzyme Rh_DypB for aflatoxin reduction in feed.

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1. Introduction

Aflatoxins (AFs) are toxic metabolites produced by *Aspergillus flavus* and *A. parasiticus* along with 11 other uncommon species of *Aspergillus*. Aflatoxin B_1 (AFB_1) is highly toxic to domestic animals and is a potent human carcinogen (World Health Organization, 2019).

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Abbreviations

AFs	Aflatoxins
AFB_1	Aflatoxin B_1
AFM_1	Aflatoxin M_1
Rh_DypB	Dye-decolorizing peroxidase type B
ABTS	2,2-azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid]

In food-insecure countries with subtropical climates or that are subject to drought conditions, crops like maize and groundnuts are prone to aflatoxin contamination. Managing this risk requires an integrated approach that spans the management of fungal contamination in the field, in storage and, where possible, practical post-harvest reduction strategies (Leslie and Logrieco, 2014). More than 500 million people, mainly in Africa, are chronically exposed to aflatoxin. This contributes to child stunting, increases the rate of liver cancer and can result in child mortality (Doerge et al., 2018; McMillan et al., 2018; Wild et al., 2015).

There is, however, an important additional negative impact of aflatoxin on human health, namely the decrease in the amount of animal proteins available for food. In many developing countries where aflatoxin is a chronic problem, the poorest quality grain (where it can be spared) is used for animal feed. Poultry species suffer from reductions in growth rate, increased susceptibility to disease, and decreased egg production. In cattle, aflatoxin causes liver and kidney damage, and reduces milk production (Eaton et al., 2010; Pitt et al. 2012). Thus, the use of aflatoxin-contaminated feed contributes to another serious problem in sub-Saharan Africa, i.e. Protein Energy Malnutrition (Bhutta et al., 2017). A practical technique to reduce aflatoxin in highly contaminated maize for feed would make an important contribution to public health in Africa.

Biological methods, such as enzymatic biotransformation, can reduce AFs concentrations in food commodities through a mild and environmental friendly approach. Many enzymes have been recognized as being capable of degrading mycotoxins, such as peroxidases and laccases, recently reviewed (Loi et al., 2017). Laccases have medium to low redox potential ($\leq$ 0.8 V) and may need redox mediators to efficiently degrade mycotoxins (Loi et al., 2018), while peroxidases have higher redox potential ($\geq$ 1.0 V), and are efficient oxidants by themselves. These enzymes are heme containing peroxidases requiring H_2O_2 to oxidize methoxylated aromatics, antraquinones, phenolics and non-phenolics lignin model compounds (Pollegioni et al., 2015).

Peroxidases are known to act on mycotoxins (including AFB_1) (Marimón Sibaja et al., 2019; Yehia, 2014; Tripathi and Mishra, 2011; Chitrangada and Mishra, 2000), although the transformation products and their associated toxicities have been poorly investigated. Oxidation of AFB_1 may result in a more toxic (carcinogenic, according to IARC) metabolite, i.e. 8,9-epoxy-AFB_1. which is the *in vivo* product of cytochrome P450 isozymes (CYP3A4 and CYP1A2) (Rushing and Selim, 2019) as well as other peroxidases (Wang et al., 2019; Wang et al., 2011). The identification of the degradation product is, therefore, a crucial issue.

In preliminary experiments we evaluated the ability of two laccases from *Bacillus licheniformis* and *Trametes versicolor*, two manganese peroxidases from *Bjerkandera adusta* and *Phlebia* sp. Nfb19 (Tonin et al., 2017), and the recombinant N246A variant of dye-decolorizing DypB (Rh_DypB) from *Rhodococcus jostii* (Vignali et al., 2018) to degrade AFB_1. Transformation of the mycotoxin was monitored qualitatively by a simple and rapid colorimetric assay (Tonin et al., 2017). Noteworthy, Rh_DypB showed the highest mycotoxin degradation activity and demonstrated to combine different dye-decolorizing and oxidative activities in a single enzyme, and to have a high yield of recombinant production (up to 100 mg enzyme/L fermentation broth) (Vignali et al., 2018).

The purpose of this study was to investigate the *in vitro* biotransformation of AFB_1 by a recombinant Rh_DypB; the enzymatic reaction was studied using various experimental conditions and the kinetic parameters were assessed. Finally, the reaction products were characterized by mass spectrometry analysis.

2. Materials and methods

2.1. Chemicals and AFB_1 standard preparation

2,2-Azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) (catalogue number A11557), H_2O_2 (catalogue number H1009), AFB_1 standard (catalogue number 32754, purity > 99%) were purchased from Sigma Aldrich Milan, Italy. Regenerated cellulose (RC) membranes 0.2 mm filters (catalogue number 656102) were obtained from Alltech Italia-Grace Division (Milano, Italy). All solvents (HPLC grade) were purchased from J. T. Baker Inc. (Deventer, The Netherlands). Ultrapure water was produced by a Milli-Q system (Millipore, Bedford, MA, USA).

AFB_1 (Sigma Aldrich, Milan, Italy) was dissolved in toluene:acetonitrile (ACN) (9:1, v/v) to prepare a AFB_1 stock solution (100 μg/mL). The exact concentration of AFB_1 was determined according to Association of Official Analytical Chemists (2000).

2.2. Rh_DypB production and purification

Rh_DypB was produced from BL21 (DE3) *Escherichia coli* cells (catalogue number 69450, Merck Millipore) transformed with pET24-Rh_DypB expression plasmid (Vignali et al., 2018). Recombinant cells were grown under aerobic conditions at 37 °C in Terrific Broth medium (24 g/L yeast extract, 12 g/L bacto-tryptone, 8 mL/L glycerol, 9.4 g/L, K_2HPO_4, 2.2 g/L KH_2PO_4) and induced adding 0.25 mM IPTG and 0.25 g/L hemin chloride at an $OD_{600nm} \approx 1.0$; the highest Rh_DypB expression was achieved under microaerobic conditions. Purification was performed using an HiTrap chelating affinity column (catalogue number 17040901, GE Healthcare, Milano, Italy), previously loaded with 100 mM $NiCl_2$. Column equilibration was performed with 20 mM MOPS buffer, pH 7.5 added with 80 mM NaCl and 5% (v/v) glycerol. Rh_DypB was eluted with the same buffer with addition of 500 mM imidazole, which was then removed by a gel-permeation chromatography (PD10 column, GE Healthcare). The obtained Rh_DypB stock solution (5 U/mg in 50 mM sodium malonate, pH 6.0, 2 mM $MnCl_2$, 0.1 mM H_2O_2, 25 °C) was stored at −20 °C until use.

2.3. Activity measurements

Enzyme activity was measured spectrophotometrically, as reported by Vignali et al. (2018). Briefly, enzyme activity was assayed in 50 mM sodium malonate buffer, pH 6.0 added with 2 mM $MnCl_2$, 0.1 mM H_2O_2 and 1 mM ABTS from the absorbance change at 420 nm recorded in the first minute of measurement ($\epsilon_{420nm} = 36{,}000\ M^{-1}cm^{-1}$). One unit of activity was defined as the amount of enzyme which consumed 1 μmol of substrate in 1 min at 25 °C.

2.4. Aflatoxin B_1 degradation assays with Rh_DypB and kinetic measurements

In order to study and optimize AFB_1 degradation upon enzymatic treatment four different experiments (Table 1, A-D) were

performed. Starting from the already optimized condition (A, Table 1) for Rh_DypB (Vignali et al., 2018), H_2O_2 and enzyme adjustments were made to counteract H_2O_2 consumption, spontaneous decomposition or enzyme denaturation during the assay. AFB_1 degradation assays were carried out in 50 mM sodium malonate buffer, pH 6.0 containing 2 mM $MnCl_2$ and AFB_1 (1 μg/mL). The reaction mixture contained different H_2O_2 and enzyme concentrations, as reported in Table 1.

In control samples, the enzyme solution was replaced by an equal amount of buffer. After gentle mixing, samples were incubated at 25 °C under shaking (150 rpm) and analyzed after 3, 6, 18, 24, 48, 72 and 96 h of reaction. Residual AFB_1 was quantified by HPLC as reported by Loi et al. (2016), and analyzed by high-resolution LC-MS/MS to identify AFB_1 degradation products as described in section 2.5.

Kinetic parameters, K_m and V_{max}, were determined using 0.1 U/mL of Rh_DypB, and increasing AFB_1 concentrations (0.1, 0.33, 0.8, 1.33 and 3 μg/mL) using the amount of substrate converted after 24 h.

2.5. High resolution LC-MS analyses of AFB_1 degradation and characterization of transformation products

The enzymatic reactions with AFB_1 (Table 1) were quenched by adding 200 μL of cold methanol (to denature Rh_DypB) to 100 μL of reaction mixture and incubated in ice for 10 min. Samples were then centrifuged at 9000 g for 5 min at 4 °C to remove precipitated protein and the supernatant was transferred to a HPLC vial.

All samples were analyzed using a Q-Exactive™ Quadrupole Orbitrap mass spectrometer (Thermo Scientific™) coupled to an Agilent 1290 high-performance liquid chromatography (HPLC) system. AFB_1 and transformation products were resolved on a Zorbax Eclipse Plus RRHD C18 column (2.1 × 50 mm, 1.8 μm; catalogue number 959757-902, Agilent) maintained at 35 °C. The mobile phase was water with 0.1% formic acid (A), and acetonitrile with 0.1% formic acid (B) (Optima grade, Fisher Scientific™, Lawn, NJ, USA). Mobile phase B was held at 0% for 30 s, before increasing to 100% over 3 min. B was then held at 100% for 2.5 min before returning to 0% over 30 s. Injections of 6 μL were used with a flow rate of 0.3 mL/min. The following conditions were used for positive HESI: capillary voltage, 3.9 kV; capillary temperature, 400 °C; sheath gas, 17.00 units; auxiliary gas, 8.00 units; probe heater temperature, 450 °C; S-Lens RF level, 45.00. Samples were analyzed using a data-dependent acquisition (DDA) experiment comprised of a full MS scan at 17,500 resolution over a scan range of 70–1000 *m/z*; automatic gain control (AGC) target and maximum injection time (max IT) was 3×10^6 and 64 ms, respectively. The five highest intensity ions from the full scan (excluding isotopes) were sequentially selected using a 1.0 *m/z* isolation window and analyzed at resolution of 17,500; AGC target, 1×10^6; max IT, 64 ms; normalized collision energy (NCE) 50; threshold intensity 1.5×10^5; and dynamic exclusion of 5 s.

2.6. Statistical analyses

Data were expressed as the mean of three replicates ± standard deviation (SD) of at least two independent experiments. Results were analyzed through ANOVA and Student's t-test (paired comparison) performed using STATISTICA software for windows, ver. 7 (Statsoft, Tulsa, Okhla). Differences between samples and relative control were considered significant for a P value < 0.05.

3. Results and discussion

Rh_DypB capability of degrading AFB_1 was tested *in vitro* and optimized using different experimental conditions and kinetic parameters were estimated. Toxin reduction was evaluated by HPLC analyses. To assess whether the enzymatic reaction resulted in the production of less toxic metabolites (i.e. AFB_1 was detoxified), samples were analyzed by mass spectrometry and the reaction products identified.

3.1. Time course degradation of aflatoxin B_1 by Rh_DypB

AFB_1 stability was assessed in 50 mM sodium malonate buffer, pH6 containing 0.1 mM H_2O_2 and 2 mM $MnCl_2$, as well as in samples added with 0.1 mM H_2O_2. AFB_1 was stable in all control samples during the whole extent of the assay (96 h) (recovery 100 ± 0.11%). H_2O_2 treatment has been reported to chemically modify AFB_1, but at higher concentrations than the one used in this study (0.06–2 M vs 0.1 mM) (Karlovsky et al., 2016).

The time course of AFB_1 degradation by Rh_DypB is shown in Fig. 1. The efficacy of a standard degradation assay (Table 1, A) was compared using various experimental conditions, which included the addition of aliquots of 0.1 mM H_2O_2 (Table 1, B), aliquots of both 0.1 mM H_2O_2 and 0.1 U/mL of Rh_DypB (Table 1, C) or higher enzyme (0.5 U/mL) and H_2O_2 (0.2 mM) concentrations (Table 1, D).

In all conditions tested, Rh_DypB was effective at modifying AFB_1, following an exponential decay ($R^2 = 0.9918$; 0.9818; 0.9767; 0.9625). The highest rate of conversion occurred in the first 18 h of assay. After 3 and 6 h the modification was higher under the standard conditions (P < 0.01). Regardless of H_2O_2 and Rh_DypB addition, the concentration of AFB_1 was halved within 24 h (51–54%), and then slowly, but continuously removed. AFB_1 biotransformation reached 77% in the standard conditions (A) and 89% with the re-addition of H_2O_2 (B), while, an almost complete biotransformation (96%) was reached when both H_2O_2 and Rh_DypB were added to the reaction mix. The use of higher enzyme concentrations and H_2O_2 amounts (D) did not lead to an increased modification at all tested time points; the biotransformation reached only 71% after 96 h.

H_2O_2 addition was not effective in increasing AFB_1 degradation, likely because it was present in large excess at the beginning of the reaction. Likewise, Rh_DypB re-addition did not significantly increase mycotoxin degradation. The only exception is apparent at 96 h, probably due to enzyme inactivation at long reaction times (C, Fig. 1).

It is worth noting that a high aflatoxin B_1 concentration (1 μg/mL) was used for the experimental design. Although aflatoxins concentration in food is usually lower (μg/kg range), the efficiency of the enzymatic degradation may be reduced under real conditions, depending on matrix composition, humidity, enzyme

Table 1
Aflatoxin B_1 degradation assays parameters.

Assay	H_2O_2 (mM)	Rh_DypB (U/mL)
A	0.1	0.1
B	0.1, aliquots added every 2 h, 4 times a day	0.1
C	0.1, aliquots added every 2 h, 4 times a day	0.1 aliquots added every 24 h
D	0.2	0.5

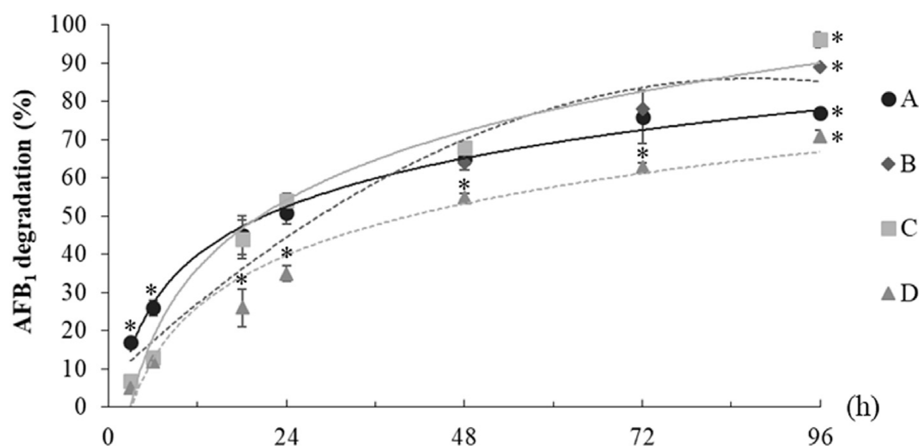

Fig. 1. Time course of biotransformation of AFB_1 by Rh_DypB in sodium malonate buffer 50 mM pH 6, 2 mM $MnCl_2$. A-D. conditions were reported in Table 1. Error bars represent the standard deviation for three independent replicates of two independent experiments. Asterisks (*) indicate a statistically significant difference between samples at the same timepoint ($P < 0.05$).

application etc. In addition, natural contamination may appear as "hot spots"; some parts may appear uncontaminated, while other show extremely high contamination levels. Therefore, although *in vitro*, the results of this study are meaningful and this enzymatic method might be an effective tool to counteract aflatoxins contamination in a real context. In previous studies, a fungal manganese dependent peroxidase from *Pleurotus ostreatus* (Yehia, 2014), a native (Wang et al., 2011) and a commercial (Marimón Sibaja et al., 2019) horse radish peroxidase and a peroxidase extracted from *Alium sativum* (Tripathi and Mishra, 2011) were shown to degrade AFB_1 *in vitro* up to 90%, 57%, 97% and 70%, respectively. Direct comparisons between these studies and our results is not feasible since we used lower concentrations of enzyme and H_2O_2, along with higher amounts of AFB_1. For instance, Marimon-Sibaja and colleagues et al. (2019) used an enzyme/AFB_1 ratio and a H_2O_2 concentration 200 and 20 times higher, respectively, than the one used in this study. A total of 0.4 U/mL were used by Yehia (2014) to obtain a percentage of degradation after 24 h of reaction similar to that reported in our study but only using 0.003 μg/mL of AFB_1 (Yehia, 2014). The findings of our study may have a high impact also for the applicability and sustainability of the enzymatic detoxification process. A first important point is that Rh_DypB is a recombinant enzyme produced in fairly large amounts (100 mg/L). Secondly, the process is effective at low H_2O_2 and enzyme amounts. These points are of main relevance to guarantee the economic sustainability of this biotransformation method. In addition, using mild conditions and low enzyme and H_2O_2 dosages will limit the possible side reactions in a complex material, such as contaminated vegetable commodities.

3.2. Kinetic measurements

The kinetic parameters of Rh_DypB on AFB_1 were determined up to 3.5 μg/mL to ensure a homogeneous mycotoxin concentration in aqueous solution (the maximal mycotoxin concentration used is limited by the poor solubility in aqueous solution, ≤ 10 μg/mL, and the need to avoid interferences from the solvents used for mycotoxin solubilization). The experimental data were fitted using a classical Michaelis-Menten equation as shown in Fig. 2. In the substrate range assayed, the plot is rather linear, indicating that the substrate concentration is far from saturating the enzyme. A maximal rate of ≈0.002 (0.0021 ± 0.0005) μg/mL per min and a K_m of ≈3.2 (3.2 ± 0.6) μg/mL were estimated.

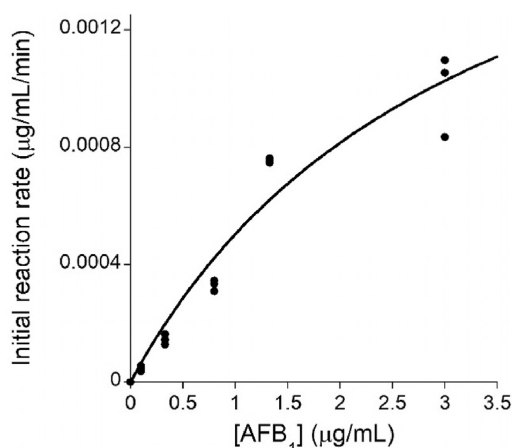

Fig. 2. Michaelis-Menten plot of the rate of conversion after 24 h vs. AFB_1 concentration for 0.1 U/mL Rh_DypB using 0.1 mM H_2O_2 in 50 mM sodium malonate buffer, pH 6.0, at 25 °C.

3.3. High resolution LC-MS/MS analyses of AFB_1 degradation products

AFB_1 showed good ionization efficiency in positive ion mode; the analyte was observed as both the $[M+H]^+$ and $[M+Na]^+$ molecular ion *m/z* 313.0706 and 335.0526.

The ability of Rh_DypB to degrade AFB_1 was also confirmed by high resolution LC-MS analysis (Fig. 3). Following 48 h of reaction at 25 °C of AFB_1 under assay condition A, a marked reduction (61%) of AFB_1 was measured, in accordance with the results obtained by HPLC analyses.

The total ion current chromatograms of the control and Rh_DypB samples were compared for the occurrences of additional peaks that could represent the chemical product of the AFB_1 reaction (Fig. 4). An additional distinct peak (marked with an asterisk in Fig. 4) was observed in the Rh_DypB sample, which was not detected in the control and in reference AFB_1.

Closer inspection of the unknown peak at retention time 2.83 revealed a *m/z* of 351.0475, corresponding to a formula of a sodiated AFB_1 metabolite $[C_{17}H_{12}O_7 + Na]^+$ (−0.01 ppm). This formula represents the addition of a single oxygen to AFB_1 and has the same formula as the known compounds: AFG_1, AFM_1, AFQ_1 and 8,9-AFB_1

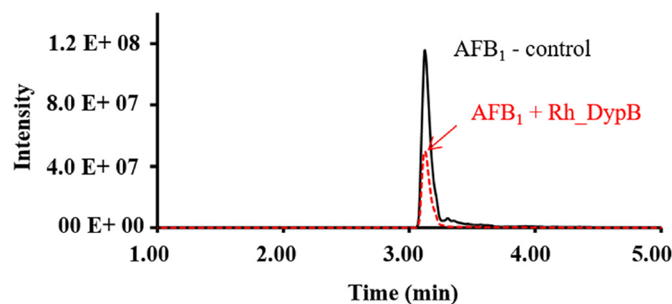

Fig. 3. Extracted ion current chromatograms of aflatoxin B_1 (AFB_1) following 48 h control treatment (black) or Rh_DypB treatment (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

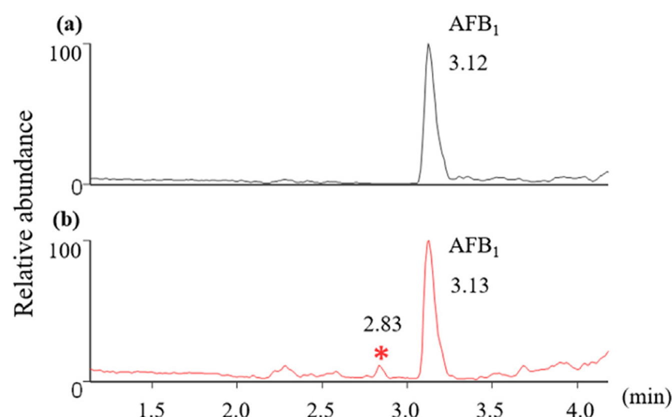

Fig. 4. Total ion chromatograms of (a) AFB_1 control and (b) AFB1+DypB after 48 h of incubation.

epoxide. Comparing the MS/MS spectrum of the unknown AF metabolite to equivalent spectra of AFG_1, AFM_1 (https://massbank.eu/: AC000034, AC000037, AC000046, AC000049), allowed exclusion of AFM_1 and AFG_1 (Fig. 5). Indeed, 8,9-AFB_1 epoxide is known to undergo rapid hydrolysis to AFB_1-dihydrodiol (Johnson et al., 1996), indicating that it is not the observed product.

The unknown Rh_DypB reaction product had a diagnostic product ion at m/z 141.0182, corresponding to a formula of $C_6H_5O_4^+$. This product ion suggests that the additional oxygen has been added to the ring A portion of the molecule. The fluorescence of the assay mixture did not decrease over time (data not shown), suggesting that adding oxygen did not disrupt the double bond of ring A, one of the determinant chemical features for AFs fluorescence (Vazquez et al., 2010). AFQ_1 is a known AFB_1 metabolite, which contains a hydroxyl group on the 3α position of ring A. Although this compound is no longer commercially available, the MS/MS spectra was generously provided by Dr. De Boevre (see Supplementary Fig. 1).

Although it was not possible to fully compare the MS/MS spectra obtained in this study (acquired on a Thermo Q-Exactive Orbitrap) with the spectra acquired on a Waters Synapt TOF, there are several diagnostic ions that match between the spectra, notably, m/z 283.0599, 206.0574 and 177.0547. Based on these, we concluded that it is highly likely that AFB_1 was hydroxylated by Rh_DypB into AFQ_1.

Any proposed enzymatic treatment of mycotoxins, especially AFB_1, requires careful identification of the reaction products, as they themselves may be equally or even more toxic than the original toxin. For example, the carcinogenicity of AFB_1 is primarily a result of the exo 8,9-epoxyAFB_1, which forms adducts with DNA, reacting with the guanyl N7 (Doerge et al., 2018). Moreover, the identification of the AFB_1 reaction products can be a difficult task since AFB_1 possesses a number of sites where enzymes can act (Bonomo et al., 2017). AFB_1 degradation products by peroxidase were questioned

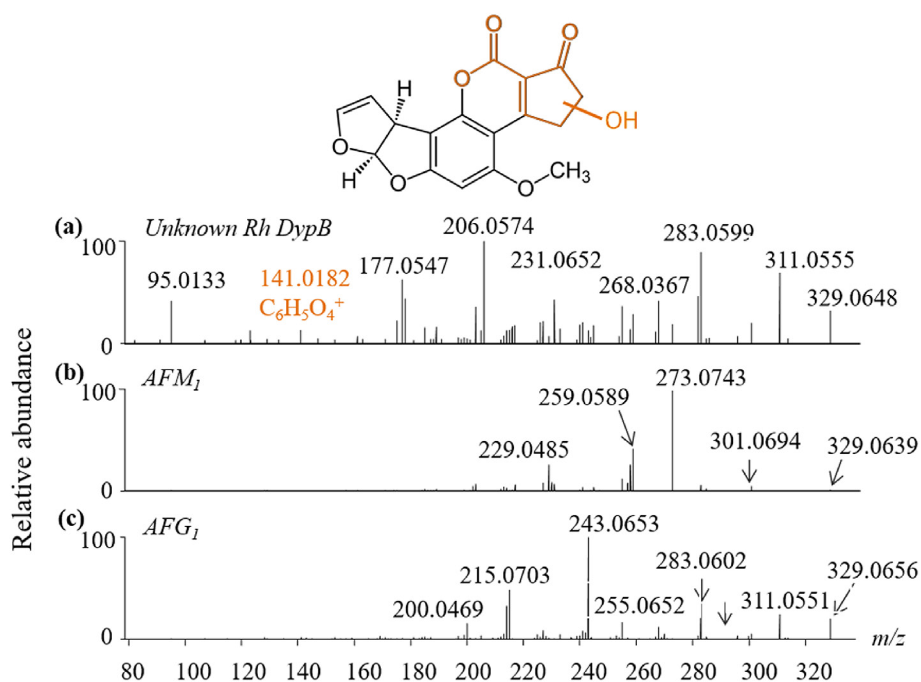

Fig. 5. MS/MS spectra of (a) unknown Rh_DypB product, (b) AFM_1 and (c) AFG_1. Arrows indicate diagnostic product ions.

since the late 70s by Doyle and Marth (1979) and more recently by Wang et al. (2011). In the latter case, oxidation to 8,9-epoxyAFB_1 leading to AFB_1-8,9-dihydrodiol (the rapid hydrolysis product of 8,9-AFB_1 epoxide) was found. In humans, four major metabolic pathways have been described for AFB_1: epoxidation to 8,9-epoxyAFB_1, ketoreduction to aflatoxicol (AFL), hydroxylation to AFM_1, and AFQ_1, and demethylation to AFP_1 (Deng et al., 2018). This wide variety of possible products is due to the fact that peroxidase can catalyze different reactions, including breaking carbon-carbon bonds, benzylic oxidative cleavage, ortho-demethylation, N-methylation, epoxidation and hydroxylation.

In our study, after Rh_DypB biotransformation, neither 8,9-AFB_1 epoxide nor AFB_1-8,9-dihydrodiol were detected by LC-MS/MS, suggesting that the C–H hydroxylation pathway is favored compared to the epoxidation of the alkene. Oxidative demethylation was also excluded, as no stable demethylated AFB_1 was found. This finding is extremely important because we can exclude the production of 8,9-epoxy-AFB_1, the more toxic oxidation product of cytochromes and other peroxidases. AFQ_1 was the main product. However, the presence of small quantities of low molecular mass compounds cannot be excluded.

Hsieh et al. (1974) reported that AFQ_1 was an order of magnitude less toxic than AFB_1 in the chicken embryo test and suggested that it was not mutagenic in an Ames assay. However, a re-analysis of these data indicated a mutagenic activity of AFQ_1 (Campbell and Hayes, 1976; Eaton and Gallagher, 1994). Based on available data, AFQ_1 has a lower acute toxicity and mutagenicity than AFB_1. In rainbow trout, AFQ_1 toxicity is ~1% of AFB_1, noting that rainbow trout is generally considered the most sensitive species to AFB_1 (Eaton and Gallagher, 1994). In mammals, glucuronide and sulphate conjugates are formed prior to the excretion of AFQ_1 in urine and feces, thus providing an effective detoxification pathway (Eaton et al., 2010).

4. Conclusions

Rh_DypB peroxidase proved to be an efficient AFB_1 biotransformation agent, being effective at low enzyme and H_2O_2 dosages and catalyzing the hydroxylation to AFQ_1. Up to 96% of bioconversion was obtained after 96 h of reaction with only 0.1 U/mL of Rh_DypB and 0.1 mM of H_2O_2. Elucidating that Rh_DypB does not catalyze the epoxidation of AFB_1, but its hydroxylation to a less toxic metabolite, AFQ_1, is a crucial step for the development of a true and safe detoxification process using peroxidases. This application could be of great interest for the treatment of commodities highly contaminated with AFB_1 and intended as feed. The main limitation of this study is the lack of evaluation of the enzyme's performance on real matrices and under operational conditions that can significantly affect mycotoxin bioconversion. Therefore, further studies are needed to validate this method in natural contaminated matrixes. Nonetheless, the results presented in this study greatly contribute to expanding the current knowledge on AFB_1 biotransformation by Rh_DypB, its reaction products and the range of possible applications of this versatile biocatalyst.

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.

CRediT authorship contribution statement

Martina Loi: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Justin B. Renaud:** Methodology, Investigation, Writing - review & editing. **Elena Rosini:** Methodology, Writing - review & editing. **Loredano Pollegioni:** Methodology, Writing - review & editing. **Elisa Vignali:** Investigation. **Miriam Haidukowski:** Investigation, Methodology. **Mark W. Sumarah:** Supervision, Writing - review & editing. **Antonio F. Logrieco:** Supervision, Funding acquisition, Writing - review & editing. **Giuseppina Mulè:** Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.126296.

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