Decontamination of Fumonisin B$_1$ in maize grain by *Pleurotus eryngii* and antioxidant enzymes

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Summary. Fumonisin B$_1$ (FB$_1$) is among the most common mycotoxins found in maize kernels and maize products worldwide. The microbiological process of detoxification and transformation of toxic organic pollutants is a promising method for foodstuffs decontamination. Some basidiomycetes, such as the *Pleurotus eryngii* species complex, include several important commercial edible varieties that can detoxify polycyclic organic compounds and a range of wastes and pollutants. We investigated the potential role of *P. eryngii*, one of the most consumed mushrooms, in the decontamination of FB$_1$ in maize. In addition, selected antioxidant enzymes, (soluble peroxidase (POD), catalase (CAT) and ascorbate peroxidase), primarily involved in control of cell hydrogen peroxide levels, and lignin degradation, were analyzed, to evaluate their contributions to the molecular mechanisms of FB$_1$ by *P. eryngii*. FB$_1$ decontamination by *P. eryngii* and involvement of CAT and POD enzymes in the control of toxic decontamination levels of H$_2$O$_2$ were demonstrated. A consistent reduction of FB$_1$ was observed at different incubation times. The average decrease levels of FB$_1$, with respect to the control cultures, ranged from 45 to 61% (RSD < 15%). This study is a possible eco-friendly approach to reducing this mycotoxin in the feed supply chains.

Key words: fungal strains, fumonisin B$_1$, hydrogen peroxide, decontamination, mycotoxins.

Introduction

Mycotoxins are secondary fungal metabolites that contaminate cereals crops and stored food or feed, and these compounds can cause adverse effects on humans, animals, and crops, causing severe economic losses (Bottalico and Perrone, 2002; Bennett and Klich, 2003). Fumonisin B$_1$ is among the most important secondary metabolites produced by *Fusarium* spp., mainly *F. verticillioides* and *F. proliferatum*, which frequently contaminate maize and maize products worldwide (Placinta *et al.*, 1999; JEFCA, 2001; Soriano and Dragacci, 2004; de la Campa *et al.*, 2005). The International Agency for Research on Cancer evaluated FB$_1$ as a group 2B carcinogen (IARC, 2002), and the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) and the Scientific Committee on Food of the European Commission (SCF) established a group tolerable daily intake (TDI) of 2 mg kg^{-1} body weight for FB$_1$, alone or in combination with other fumonisins (JECFA, 2001; SCF, 2003). In 2007 the European Commission set limits for total fumonisins (FB$_1$ and FB$_2$) at 1000 mg kg^{-1} for maize grain and maize-based foods for direct human consumption, and at 800 mg kg^{-1} for maize-based breakfast cereals and snacks (European Commission 2007). Several studies on the toxicity of FB$_1$ indicated that this mycotoxin could cause leukoencephalomalacia in horses, pulmonary oedema in pigs, and nephrotoxicity and hepatotoxicity in several animals (Marasas *et al.*, 2005; Desjardins, 2006). Epidemiologic studies have also shown that FB$_1$ can be associated with increased incidence of human oesophageal cancer and neural tube defects (Yoshizawa *et al.*, 1994).

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Although several preventive strategies to reduce the formation of mycotoxins are applied, it is sometimes difficult to control mycotoxin contamination of grain in the field and during storage (Awad *et al.*, 2010). In recent years, many physical and chemical approaches have been applied for detoxifying mycotoxin-contaminated food and feed. Nevertheless, only few of these have been accepted for practical use.

Mycoremediation is now considered as a promising approach as a novel and efficient strategy for degradation and transformation of toxic organic pollutants. Basidiomycota are among the most potent organisms for to biodegrading and detoxifying a wide range of chemicals and environmental pollutants.

White rot basidiomycetes, such as *Pleurotus* spp., are functional foods in many countries, and play important roles in many biotechnological processes (Martínez *et al.*, 2000; Stajic *et al.*, 2009). These fungi are sources of enzymes such as oxygenases (mono and dioxygenase), laccase, peroxidases (lignin peroxidase, manganese peroxidase, and versatile peroxidase), and hydrolases (lipase, cellulose, and protease) that degrade a variety of polycyclic organic compounds and detoxify a wide range of wastes and pollutants (Hofrichter, 2002; Rodríguez *et al.*, 2004; Kristanti *et al.*, 2011; Mouhamadou *et al.*, 2013) and industrial dyes (Heinfling *et al.*, 1998). These organisms are useful for bioremediation of soil and industrial water.

The present study assessed different isolates of *P. eryngii*, a typical Apulian fungal variety and one of the most consumed mushrooms, for their degrading effects on FB_1 contaminated maize grain. The molecular mechanisms of FB_1 decontamination by *P. eryngii* were investigated by analyzing selected antioxidant enzymes, including soluble peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX), that are primarily involved in controlling hydrogen peroxide levels in cell and in the degradation of lignin.

Materials and methods

Mycological analyses

Strains

Four strains of *P. eryngii* (ITEM 13681, ITEM 13682, ITEM 13688 and ITEM 17015) and a strain of *F. verticilliodes* (ITEM 3418) were obtained from the ITEM microbial collection at ISPA-CNR (website: http://www.ispa.cnr.it/Collection). The *F. verticil-liodes* strain (ITEM 3418) was selected because it has previously tested positive for FB_1 production.

Preparation of contaminated maize

The substrate used for *P. eryngii* growing was obtained by inoculating maize grain with *F. verticilliodes* (ITEM 3418). Maize kernels (50 g) moistened overnight with distilled water to about 45% moisture content, were autoclaved in 250 mL capacity Erlenmeyer flasks for 30 min at 120°C. Each sample was then inoculated with 2 mL of an aqueous suspension containing approx. 10^7 conidia mL^{-1}, and shaken once daily for 3 d to distribute the inoculum. The cultures were incubated at 25°C in the dark for 20 d. The harvested culture material was dried in a forced draft oven at 60°C for 48 h, then milled to obtain a fine flour, and was stored at 4°C until used.

Preparation and inoculation of substrates

An amount equal to 8.3% (w/v) of contaminated maize flour was mixed with 300 mL of distilled water and 0.8% of agar. The substrate (MCONT) was autoclaved at 121°C for 15 min and poured into Petri dishes (9 cm diam.), and allowed it to cool. Plugs of agar (6 mm diam.) from 10-d-old cultures of the different strains of *P. eryngii,* grown on malt extract agar (MEA), were placed in the centre of Petri dishes and incubated for 12 and 20 d at 27°C. Negative controls were substrate (MCTRL) obtained in the same way, without contaminated maize flour, and were inoculated with the same test strains, while positive controls were the uninoculated MCONT substrate. Plates containing MEA (synthetic medium control) were also included to determine any differences of *P. eryngii* growth on the MCONT and MCTRL agars. Three replicates per substrate and strain for the two-incubation periods (12 and 20 d) were used.

Mycelium growth

After 12 and 20 d of incubation, all plates were assessed for mycelium growth rate, by measuring colony diameters using Vernier calipers in two orthogonal directions. The final diameter of colonies was calculated as the arithmetic mean of these two measurements.

Chemical analyses

Extraction protocol

After the end of the two incubation periods, from each plate, five agar plugs (0.8–1.0 g) were cut out

of the colony, from the centre and along a radius toward the edge. The plugs were extracted with a solution methanol/water (70:30, v/v), using a volume five times greater than the weight of each sample. Samples were placed in a shaker for 60 min at 250 rpm, and were then filtered through Whatman no. 4 filters. Two millilitres of extract were the evaporated under an air stream at ca. 50°C. The residues were each dissolved in 1 mL of acetonitrile/water (30:70, v/v) and were then filtered using spin filter tubes 0.20 µm RC filters, GRACE (Alltech).

FB₁ analyses

The HPLC/FLD determination and confirmation of FB_1 was performed according to the procedure described by De Girolamo *et al.* (2011). Fifty µL of each extract was derivatized with 50 µL of o-phtaldialdehyde (OPA) mixed for 50 s using the HPLC autosampler Agilent 1100 equipped with a binary pump, column thermostat set at 30°C. The 100 µL volume was injected by full loop at 3 min after adding the OPA reagent for fumonisins analysis. The analytical column was a Symmetry Shield RP18 150 × 4.6 mm, 5 µm (Waters) with a guard column inlet filter (0.5 µm × 3 mm diam., Rheodyne Inc.). The mobile phase consisted of a binary gradient applied as follows: the initial composition of the mobile phase 57% of (A) water/acetic acid (99:1, v/v) / 43% of (B) acetonitrile/acetic acid (99:1, v/v) was kept constant for 5 min, then solvent B was linearly increased to 54% at 21 min, then up to 58% at 25 min, and then kept constant for 5 min. The flow rate of the mobile phase was 0.8 mL min⁻¹. The fluorometric detector was set at wavelengths, ex = 335 nm, em = 440 nm. Retention time for FB_1 was about 17 min.

For FB_1 analyses, a diluted stock solution for standardisation purposes with a final concentration of 5 µg mL⁻¹ of toxins was prepared in ACN/water (1:1, v/v) by appropriate dilution of the certified calibrant solution Biopure (Romer Labs Diagnostic GmbH). Recovery assessments were performed in triplicate. Fumonisin certified calibration solution was added to 1 g of agar (MCTRL) at 1 µg g⁻¹ or 10 µg g⁻¹), and allowed to stand for 1 h at room temperature to allow solvent evaporation. The remaining sample was then analysed according to the methods described above. The calibration curve showed good linearity in the range 0.005-0.25 µg mL⁻¹. Mean recovery was 95% and the RSD was less than 5%. The LOQ of the method was 12.5 µg kg⁻¹.

Other metabolite analyses

Structural confirmation of FB_1 decontamination and identification of other possible degradation metabolites were carried out using LC-HRMS. One mL of acetonitrile/water (30:70, v/v) extract was purified through a C18 column. Sep-pak C18 Vac RC cartridges (500-mg sorbent) were purchased from Waters Corporation, previously conditioned with 5 mL of MeOH followed by 5 mL of acetonitrile/water (1:9, v/v). Sample (10 mL) was then added. The C18 column was washed with 5 mL of acetonitrile/water (1:9, v/v), and then eluted with 20 mL of ACN/H₂O (1:1, v/v). Column eluates were collected and evaporated to dryness at 50°C under nitrogen streams, then re-constituted with 1 mL of MeOH/H₂O (80:20, v/v) containing 0.5% acetic acid.

Twenty µL of each supernatant was analyzed by LC-HRMS (De Girolamo *et al.*, 2014), using a Gemini C18 150 × 2.0 mm, 5-µm (Phenomenex) preceded by a Gemini C18 guard column (4.0 × 2.0 mm, 5 µm). The mobile phase was a multistep gradient of water (solvent A) and methanol (solvent B), both containing 0.5% acetic acid. The initial composition of the mobile phase was 20% solvent B, and the eluent A was linearly decreased to 0% over 35 min. LC-HRMS analyses were performed on a benchtop single-stage mass spectrometer (Exactive™) equipped with a heated electrospray ion source (HESI II), coupled to an HPLC system Accela (Thermo Fisher Scientific).

The HESI II interface was used in the positive-ion mode, and the scan range was 50.2 to 1,003.0 mz⁻¹, with a resolution power of 100,000 full width at half maximum (FWHM). Other settings were as follows: sheath and auxiliary gas flow rates, respectively 30 and 10 arbitrary units; sweep gas, 0 arbitrary units; capillary temperature, 300°C; and capillary voltage, 4 kV. The Xcalibur software (version 2.1.0, Thermo Fisher Scientific) was used for data acquisition and processing.

Enzyme extraction and determination of enzyme activities

Mycelia grown on different growth substrates after 12 or 20 d were collected and lyophilized. Enzyme extracts were obtained using the procedure described by Lanubile *et al.* (2012). Lyophilized mycelia (60 mg per sample) were frozen in liquid nitrogen and homogenized at 4°C in 50 mM Tris-HCl (pH 7.8), 0.3 mM mannitol, 1 mM EDTA, and 10 mM MgCl₂

in a 1/3 ratio (w/v). The homogenates were centrifuged at 25,000g for 20 min at 4°C. The resulting supernatants were desalted by dialysis against 50 mM Tris-HCl pH 7.8 and stored at -80°C until used. Total proteins were determined using Bradford's (1976) method with bovine serum albumin as standard. The activities of the analyzed enzymes soluble ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6) and soluble peroxidase (POD; EC 1.11.1.7) were tested according to Paciolla *et al*. (2008) and Mastropasqua *et al*. (2012). APX was assayed using a solution containing phosphate buffer 0.1 M, pH 6.4, crude extract (100 μg of total proteins), and ascorbic acid 50 μM (final concentration). The reaction was conducted at 265 nm, using as starter of the reaction H_2O_2 160 μM (final concentration). The enzyme activity was calculated using an ε of 4.56 mM^{-1} cm^{-1}. CAT activity was measured in a reaction mixture containing phosphate buffer 0.1 M, pH 7, and crude extract (100 μg of total proteins). The reaction was conducted at 240 nm using as starter of the reaction H_2O_2 (14 mM final concentration). The enzyme activity was calculated using an ε of 7.81 mM^{-1} cm^{-1}. POD was determined in Tris-acetate buffer 50 Mm, pH 5, crude extract (100 μg of total proteins) and 4-methoxy naphthol (1 mM final concentration). The reaction was conducted at 262 nm, using as starter of the reaction H_2O_2 (100 μM final concentration). The activity was calculated using an ε of 21 mM^{-1} cm^{-1}.

Statistical analyses

All experiments were performed in triplicate and repeated at least three times. The results obtained were first analyzed by one-way analysis of variance (ANOVA), and then the Duncan's test was used as a post-hoc range test to compare the means. Mean differences were statistically considered significant at $P<0.05$. All statistical analyses were performed using STATISTICA version 6.0 (StatSoft).

Results

Influence of substrate and fungus strain on mycelium growth rates

The mycelium growth rates of different *P. eryngii* strains on various substrates are shown in Table 1. After 12 d incubation, MCTRL gave the greatest mycelium growth, while between MEA and MCONT, differences of colony diameters were related to the different strains. On the three substrates, the mycelium growth rate of ITEM 13681 and ITEM 13688 was greater ($P<0.05$) than for ITEM 13682 and ITEM 17015. After 20 d of incubation, no differences were observed for strains grown on MCTRL, while the lowest growth rate was recorded for strain ITEM 13682 on the MCONT substrate. On MEA, and at 12 d, growth rates of ITEM 13681 and ITEM 13688 were confirmed greater than ITEM 13682 and ITEM 17015.

Table 1. Average (± SD*) of 4 *P. eryngii* strain colony diameters grown on different substrates

Substrate	Period	*P. eryngii* strain			
		ITEM 13681	**ITEM 13682**	**ITEM 13688**	**ITEM 17015**
MEA	T12	25.2[b] ± 0.3	21.3[a] ± 0.3	25.3[b] ± 0.3	19.0[a] ± 0.9
MCTRL	T12	53.7[f] ± 3.3	48.5[e] ± 1.3	57.2[g] ± 2.5	44.3[d] ± 0.6
MCONT	T12	32.7[c] ± 0.8	19.0[a] ± 0.9	30.0[c] ± 3.5	26.7[b] ± 2.9
MEA	T20	84.0[d] ± 0	72.0[c] ± 6.9	82.5[d] ± 0	63.8[b] ± 1.2
MCTRL	T20	84.7[d] ± 0.6	84.5[d] ± 0.9	85.0[d] ± 0	84.5[d] ± 0.5
MCONT	T20	85.0[d] ± 0	60.3[a] ± 2.6	85.0[d] ± 0	84.5[d] ± 0.9

Means (n = 3) with different superscripts (for either T12 or T20) are significantly different ($P<0.05$).
*SD, standard deviation.
T12, after 12 days of incubation; T20, after 20 days of incubation.
MEA, malt extract agar; MCTRL, uninoculated maize substrate; MCONT, contaminated maize substrate.

Table 2. FB_1 reduction of different *P. eryngii* strains on contaminated maize substrate (MCONT) compared to the control (uninoculated substrate).

Strain	Incubation time			
	12 days		20 days	
	ppm[1] (µg/g)	% of FB_1 reduction	ppm[1] (µg/g)	% of FB_1 reduction
Control	110.1[d] ±1.9	-	105.6[d] ± 3.9	-
ITEM 13681	81.7[c] ± 10.1	26	49.2[ab] ± 1.7	53
ITEM 13682	65.9[bc] ± 12.9	40	58.1[ab] ± 8.6	45
ITEM 13688	109.7[d] ± 6.1	0	104.3[d] ± 6.1	1
ITEM 17015	104.0[d] ± 19.3	5	41.4[a] ± 0,6	61

[1] Means (n = 3) ± SD* with different superscripts are significantly different ($P<0.05$).
* SD, standard deviation.

Comparison of FB1 reduction by different *Pleurotus eryngii* strains

In general, reductions of FB_1 content were recorded after 12 and 20 d of incubation in contaminated substrate (MCONT) inoculated with *P. eryngii* strains (Table 2). Significant decreases ($P<0.05$) in FB_1 amounts compared to uninoculated controls was recorded for *P. eryngii* ITEM 13681 and ITEM 13682 after 12 d of incubation (Table 2). The average proportional reductions, ranged from 26 to 40% (RSD < 20%), and no statistically significant differences FB_1 contents were detected between these two strains.

After 20 d of incubation, all samples showed significant ($P<0.05$) decreases of mycotoxin contents, except for *P. eryngii* ITEM 13688, for which no FB_1 reduction was measured. The decreases of FB_1 levels in MCONT cultures compared to uninoculated controls ranged from 45 to 61% (RSD < 15%), and no statistically significant differences among them were observed. Moreover, for *P. eryngii* ITEM 13681 there was consistent decreased mycotoxin concentrations ($P<0.05$) at the end of the two incubation periods.

Nevertheless, in the MCONT samples inoculated with *P. eryngii* ITEM 13682, the FB_1 decrease recorded after 12 d of incubation was not different ($P>0.05$) from the FB_1 levels detected at the end of the second incubation period. Conversely, *P. eryngii* ITEM 17015 gave a significant reduction of approx. 61% in FB_1 content, during the second incubation period (12 to 20 d).

LC-HRMS analyses showed only partial hydrolysis of the fumonisin B_1 ($PHFB_1$) in contaminated maize (Figures 1 and 2). However, the absence of hydrolyzed products of fumonisin B_1 (HFB_1) in the agar cultures, confirmed by LC-HRMS, indicates that degradation did not determine the production of hydrolysed forms of FB_1 (Figure 3). In addition, the results obtained by HRMS analyses showed that $PHFB_1$ was detected not as metabolite of degradation by *P. eryngii* (Table 3), confirming only a clear and significant decreased of FB_1.

Enzyme activity in mycelia grown on different substrates

Figure 4 shows the specific activity of catalase (CAT) enzyme. At 12 d, among the strains grown on MEA, the mycelium of ITEM 17015 showed the highest activity (4.64 nmol mg^{-1}) followed by ITEM 13681 (1.6 nmol mg^{-1}), ITEM 13688 (1.25 nmol mg^{-1}) and ITEM 13682 (1 nmol mg^{-1}) (Figure 4A). A similar trend occurred in the strains growth on MEA after 20 d (Figure 4B). The colonies grown on MCTRL for 12 d showed significant decreases in CAT activity, compared to the corresponding colonies grown on MEA. The exception was for mycelium samples of ITEM 13681, in which a significant increase was observed (Figure 4A). At 20 d, generally, all the tested strains maintained the same trend as at 12 d, with except for ITEM 13681 where no difference in CAT
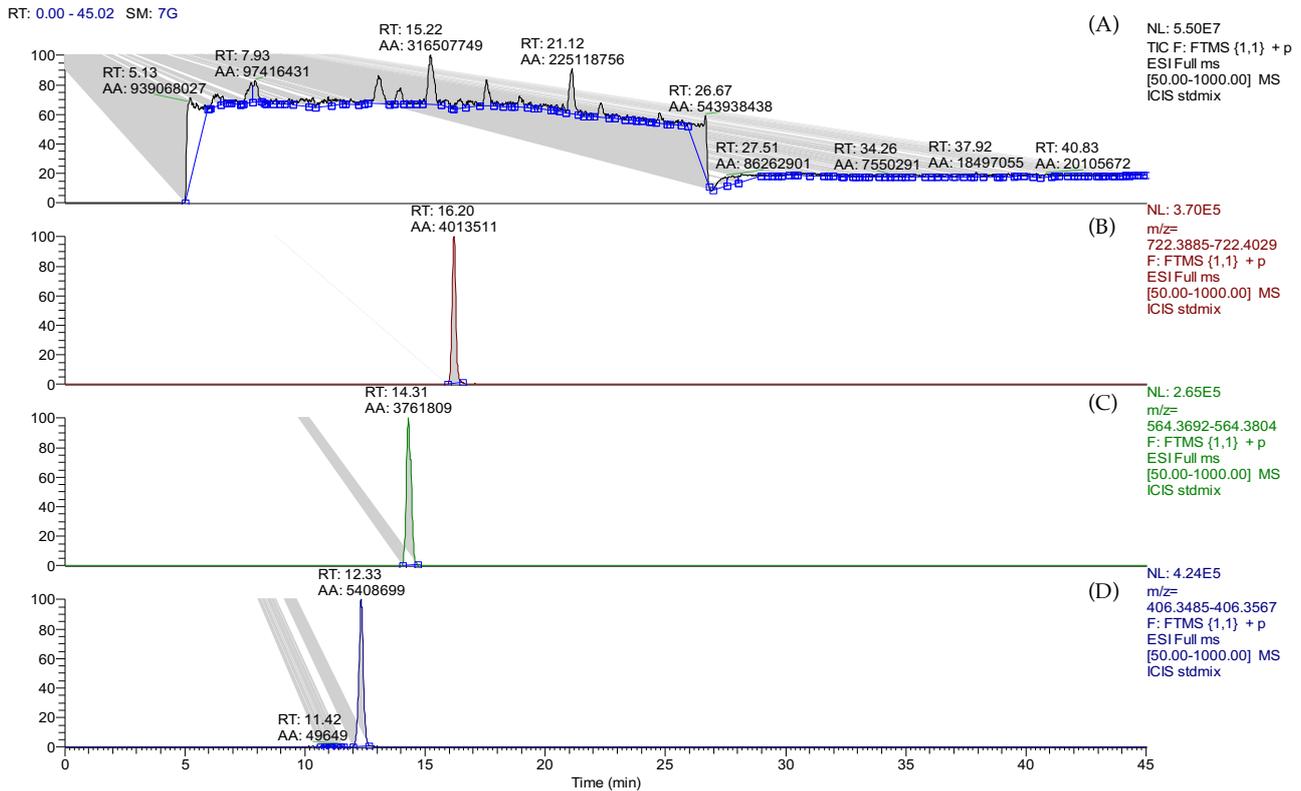
Figure 1. LC-HRMS results. (A) Total Ion Current (TIC) and extracted ion chromatograms (XIC); (B) filtered on the accurate mass of fumonisin B_1; (C) partially hydrolyzed form of FB_1 (PHFB$_1$); and (D) hydrolysed fumonisin B_1 (HFB$_1$) in standard solution.

activity was observed when grown on MCTRL or MEA (Figure 4B).

At 12 d, the mycelia of the strains ITEM 13688 and ITEM 13682 grown on contaminated maize (MCONT) substrate, gave increased ($P<0.05$) CAT activity (on average, 2.8 times greater for ITEM 13688 and 4.6 times greater for ITEM 13682) compared to colonies grown on not-contaminated maize substrate (MCTRL). There was no detected change in CAT activity for ITEM 13681 or ITEM 17015 (Figure 4A). At 20 d, different behaviour in MCONT occurred compared to the MCTRL mycelia samples, for both ITEM 13681 (decrease) and ITEM 17015 (increase) in CAT activity (Figure 4B). For the other on MCONT, the increase in activity was greater than at 12 d; in particular, the increase about 6 for ITEM 13682 and 14 times for ITEM 13588, compared to the MCTRL samples (Figure 4B).

The activities soluble peroxidase (POD) are shown in Figure 5. After 12 d on MEA, the mycelia

of each strain gave similar and low POD activities (Figure 5A), and a similar trend was detected after 20 d (Figure 5B). Increases ($P<0.05$) in POD activity occurred in MCTRL at both 12 and 20 d for all strains, as compared to MEA. The greatest activity was observed in ITEM 13682 at both time points (0.480 nmol mg^{-1} at 12 d and 0.444 nmol mg^{-1} at 20 d), and the lowest activity was measured in ITEM 17015 at 12 d (0.124 nmol mg^{-1}). In particular, at 12 d, POD activity increased in MCTRL samples compared with MEA-grown colonies by approx. 28 times in ITEM 13682, 11 times in ITEM 13688, 9 times in ITEM 13681 and 4 times in ITEM 17015. Increases after 20 d were approx. 25 times for ITEM 13682, 6 times for ITEM 17015, 4 times for ITEM 13688 and 3 times for ITEM 13681, in comparison with respective colonies grown on MEA. After growth on contaminated maize, decreases ($P<0.05$) of POD activity occurred in all strains at both 12 and 20 d, in comparison to colonies grown on MCTRL. However, the activities
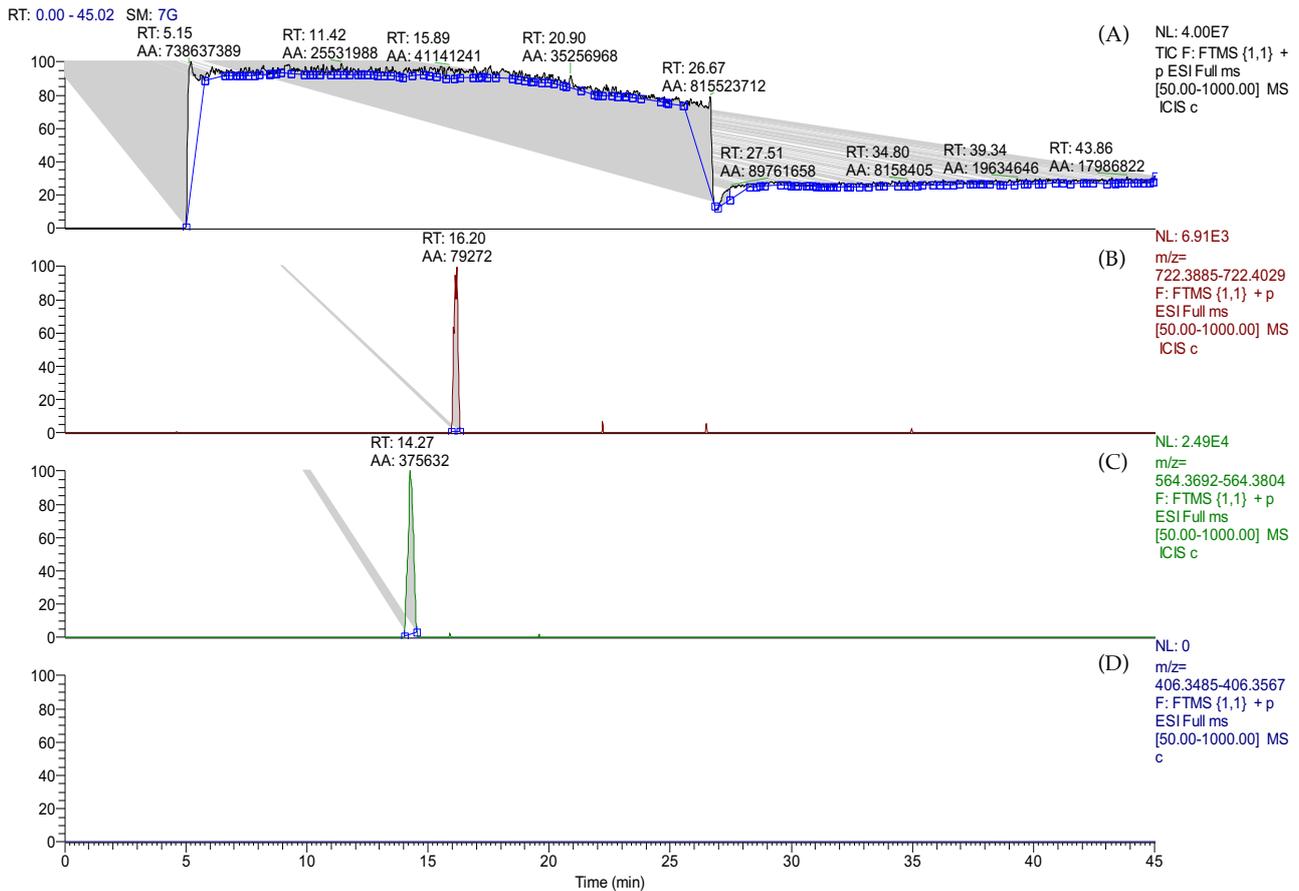
Figure 2. LC-HRMS results. (A) Total Ion Current (TIC) and extracted ion chromatograms (XIC); (B) filtered on the accurate mass of fumonisin B_1; (C) partially hydrolyzed form of FB_1 (PHFB$_1$); and (D) hydrolysed fumonisin B_1 (HFB$_1$) in control solutions without *Pleurotus*.

at 20 d were greater ($P<0.05$) for all strains grown on MEA (Figure 5B), while at 12 d the activity was greater only for ITEM 13682 and was similar for the other strains (Figure 5A).

Ascorbate peroxidase activity was not detected in these experiments (data not shown).

Discussion

Fumonisin B_1 is one among the most important mycotoxins, produced by several species of *Fusarium*, mainly *F. verticillioides* or *F. proliferatum*, which frequently occur in maize kernels and affect grain quality (Logrieco *et al.,* 2003; Voss *et al.,* 2007). The analyses of enzyme activity was carried out on *P. eryngii* mycelium obtained from colonies grown on agar contami-

nated substrates, in order simulate natural conditions and better understand the mechanisms involved in the control and regulation of mycelium growth. The results indicate suggest that the FB_1 detoxification ability of *Pleurotus eryngii* is influenced both by the strain genotypes and by the physicochemical environments in which they are growing.

The analyses of the controls at 0, 12 and 20 d showed that the toxin is stable, which allows for reproducible experiments. The different strains that we used had different capabilities for degrading FB_1.

Few microorganisms are capable of removing tricarballylate (TCA) side groups from organic molecules, which are two of the major structural groups responsible of FB_1 toxicity. Benedetti *et al.* (2006) showed that two microbial strains belonging to *Delf-*
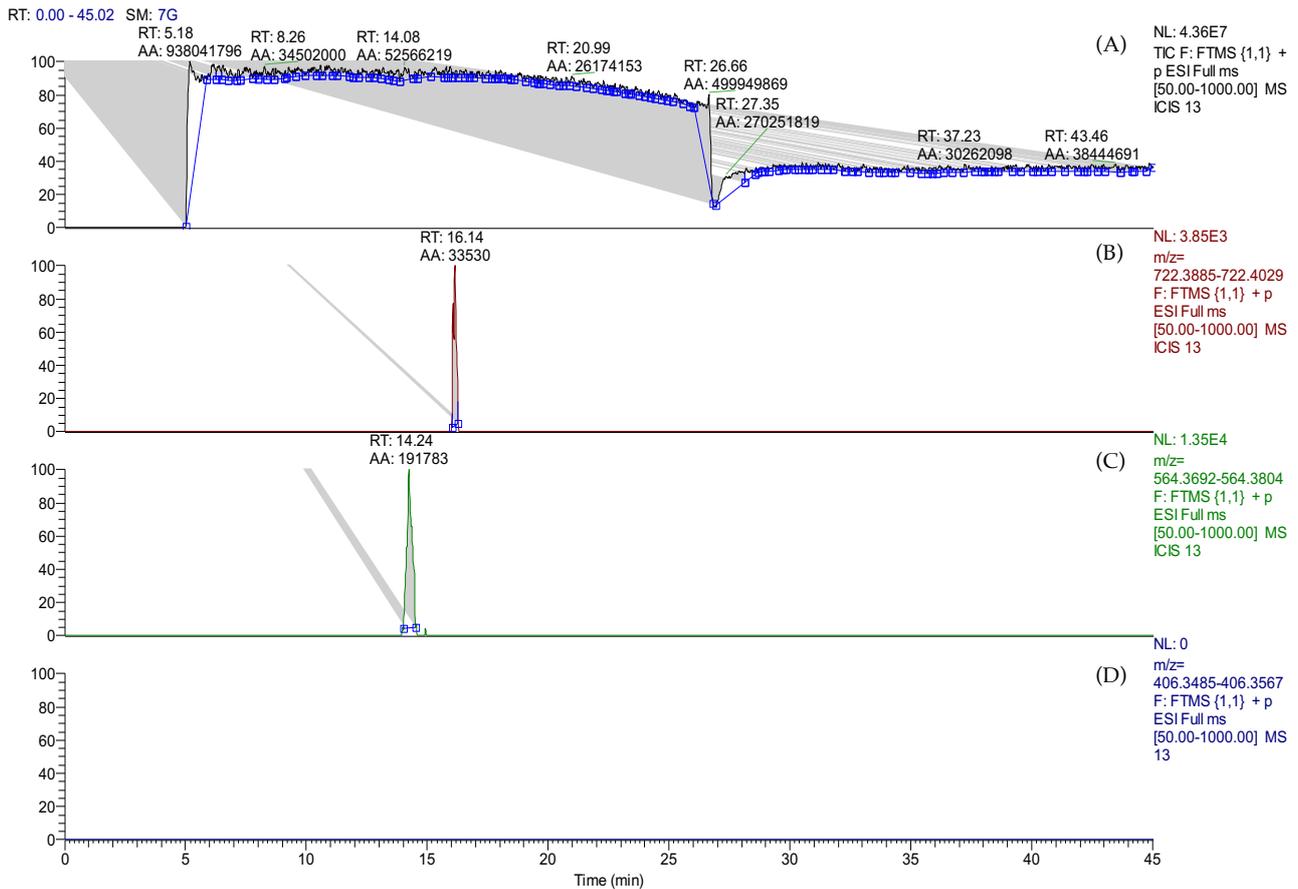
Figure 3. LC-HRMS results. (A) Total Ion Current (TIC) and extracted ion chromatograms (XIC); (B) filtered on the accurate mass of fumonisin B$_1$; (C) partially hydrolyzed form of FB$_1$ (PHFB$_1$); and (D) hydrolysed fumonisin B$_1$ (HFB$_1$) in sample ITEM 13682.

tia/*Comamonas* groups, isolated from maize field soil, gave rise to four tentative degradation products of FB$_1$ (C$_{34}$H$_{59}$NO$_{15}$): heptadecanone (C$_{17}$H$_{34}$O), isononadecene (C$_{19}$H$_{38}$), octadecenal (C$_{18}$H$_{34}$O), and eicosane (C$_{20}$H$_{42}$). These resulted from deamination of the molecules followed by reduced degradation of the aliphatic chains. In another study, *Sphingopyxis* spp. isolated from field-grown, mouldy maize kernels and stalk tissues (Duvick *et al.,* 1998) possessed two different enzymatic activities, carboxylesterase (Duvick *et al.,* 2003) and an aminotransferase (Heinl *et al.,* 2011). These were responsible for complete detoxification of FB$_1$. In the present study, FB$_1$ was not subjected to deamination or hydrolysis as determined by LC-HRMS analyses. In addition, the decontamination of FB$_1$ by *P. eryngii* was likely to be regulated by the en-

zymes controlling the steady-state level of hydrogen peroxide in fungal cells. It is well known that H$_2$O$_2$ is an important signal molecule in cells, although it is toxic if accumulated at elevated concentrations (Dat *et al.,* 2000; Quan *et al.,* 2008). In this respect, CAT, APX and POD have important roles in the cell detoxification processes, being able to remove toxic surpluses of H$_2$O$_2$. The absence of APX, however, suggests that in *P. eryngii* this enzyme is not the main detoxifying agent, as in plants kingdom where APX is ubiquitous. The presence of high CAT activity at 12 and 20 d in all strains grown on uninoculated substrate (MEA) suggests that CAT is the key enzyme in controlling the balance state of H$_2$O$_2$ in *P. eryngii*. On the other hand, our results indicated that the different strains of *P. eryngii* had different constitutive or basal

Table 3. Summary of compounds searched to identify potential degradation products by LC-HRMS with molecular formulae and accurate mass values (theoretical mass) of characteristic ions.

Compound	Molecular formulae	Theoretical mass [M+H]+
FB$_1$	C$_{34}$H$_{59}$NO$_{15}$	722.3957
PHFB$_1$	C$_{28}$H$_{53}$NO$_{10}$	564.3742
HFB$_1$	C$_{22}$H$_{47}$NO$_5$	406.3527
FA1	C$_{35}$H$_{62}$NO$_{16}$	707.5416
FC1	C$_{33}$H$_{57}$NO$_{15}$	677.4946
FP1	C$_{39}$H$_{62}$NO$_{16}$	755.5416
EFB$_1$ LA	C$_{52}$H$_{89}$NO$_{16}$	984.6254
EFB$_1$ PA	C$_{50}$H$_{89}$NO$_{16}$	960.6254
EFB$_1$ OA	C$_{52}$H$_{91}$NO$_{16}$	986.6410
N-(deossi-D-frutto-1yl)-FB$_1$	C$_{40}$H$_{69}$NO$_{20}$	884.4485
N-(carbossimetil)-FB1	C$_{36}$H$_{61}$NO$_{17}$	780.4012
Lysil-FB$_1$	C$_{43}$H$_{75}$N$_3$O$_{17}$	906.5169
FB$_1$+Metossiglucosio	C$_{50}$H$_{85}$NO$_{24}$	1022.7461
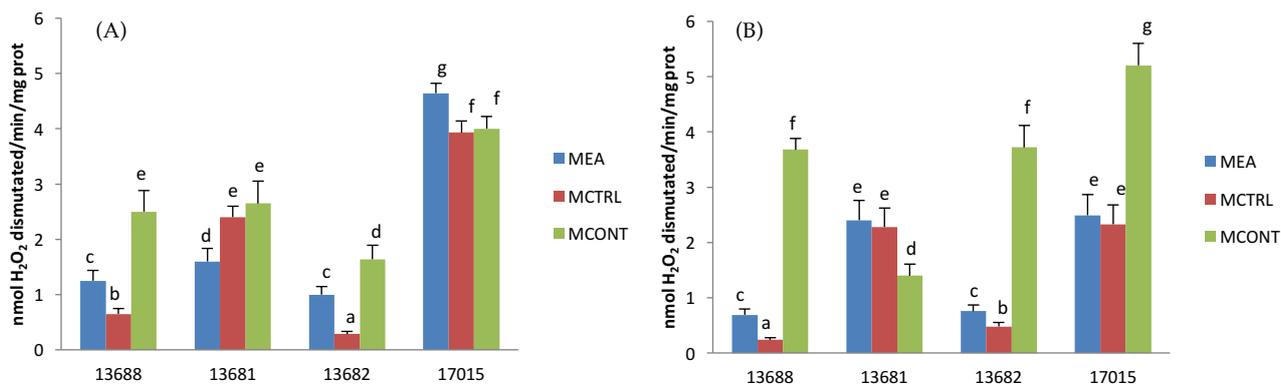
Figure 4. Catalase (CAT) activity in different strains of *Pleurotus eryngii* grown on agar (MEA), on agar containing non-contaminated maize (MCTRL) or contaminated maize (MCONT) for 12 (A) or 20 (B) d. Values represent the means of at least three replications from three independent experiments. Same letters over the columns indicate non-significant differences between treatments within each strain (*P*<0.05, Duncan's test).

POD and CAT activities, and that enzyme activities were differentially influenced by growth substrate. On MEA, the strains grown on MCTRL and MCONT media were subject to metabolic perturbations involving the CAT and POD enzymes. In particular, POD was highly induced or activated in presence of uncontaminated maize. Moreover, the significant reduction of POD activity occurring at both incubation periods in the strains grown in presence of contaminated maize, suggests that FB$_1$ is involved in the POD decay. In contrast, the increase of CAT activity generally occurring in strains grown on contaminat-
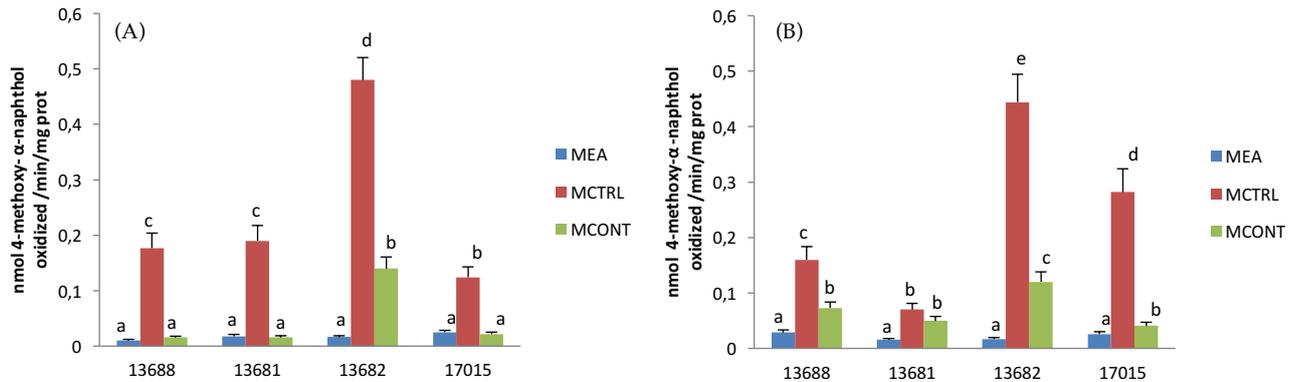
Figure 5. Soluble peroxidase (POD) activity in different strains of *Pleurotus eryngii* grown on agar (MEA), on agar containing non-contaminated maize (MCTRL), or contaminated maize (MCONT), for 12 (A) or 20 (B) d. Values represent the mean of at least three replications from three independent experiments. Same letters over the columns indicate non-significant differences between treatments within each strain (*P*<0.05, Duncan's test).

ed maize for 20 d reinforces the suggestion that this enzyme, more than POD, was capable of H_2O_2 degradation for longer periods, and played a central role against oxidative stress.

These results indicate that strains of *Pleurotus eryngii* generally possess efficient mechanisms for controlling the H_2O_2 level using CAT and POD. Because some PODs might be manganese peroxidases, we speculate that the strains tested here could be able to degrade lignin (Peláez *et al.,* 1995; Ruiz-Duenas *et al.,* 1999; Hofrichter, 2002).

It is well-known that white rot fungi, such as *A. tabescens, P. sordida, P. ostreatus, T. versicolor*, and *Peniophora* sp., have degrading capabilities of natural lignin substrates and a broad spectrum of structurally diverse toxic environmental pollutants (e.g., munitions waste, pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, bleach plant effluent, synthetic dyes, synthetic polymers, and wood preservatives) (Reddy, 1995; Pointing, 2001). The degradation activity of these basidiomycetes is usually closely related to enzyme activity of lignin peroxidases, manganese peroxidases and laccases. These are enzymes naturally involved in lignin degradation based on oxidative mechanisms, but in particular conditions they can degrade different classes of mycotoxins, including aflatoxin and ochratoxin (Engelhardt, 2002; Motomura *et al.,* 2003; Wang *et al.,* 2011). After 4 weeks in solid fermentation, *P. ostreatus* showed efficient biodegradation of ochratoxin A (OTA) and ochratoxin B (OTB).

Only 23% of the initial OTA and 3% of OTB were detected, and the intermediate products ochratoxin α and ochratoxin β were formed during the first step of degradation (Engelhardt, 2002). Fabbri *et al.* (2011) showed that semi-purified and purified exo-polysaccharides and some secreted hydrolytic enzymes (e.g., ligninolytic enzymes) from *T. versicolor* have the ability both to inhibit the biosynthesis and/or degrade different mycotoxins (aflatoxins, ochratoxin A, *Fusarium* toxins) already present in several feed and food materials such as wheat and maize seeds.

The preliminary results obtained in the present study, particularly on maize substrate contaminated by FB_1, indicate potential for this Basidiomycete to be used for control of FB_1. Our results indicate the possible use of this fungus on large scale, because of potential low cost in comparison with other preparations or the use of purified enzymes. However, understanding of the molecular characteristics of *P. eryngii* FB_1 detoxification mechanisms could help to identify the best strains for exploitation in food industry and bioremediation processes. The detoxification of FB_1 by *P. eryngii* strains could be an eco-friendly approach to reducing the levels of this mycotoxin in the waste of food supply chains as spent substrate. Moreover, further investigations using isotope labelling, are required to identify degraded products and to evaluate the possible uses of this white-rot species in bioremediation industries.

Acknowledgements

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