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Fungal Detoxification of Certain Fusarium Moniliforme Mycotoxins Using Yarrowia Lipolytica with Special Emphasis on its Possible Mechanism of Action

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Abstract: *Detoxification of mycotoxins must be taken to ensure food and feed safety. The aim of this study was to determine the influence of Yarrowia lipolytica on mycotoxin elimination and inhibition of Fusarium moniliforme growth. In this study, Y. lipolytica could remove 84.92% of Fuminosin B1 (FB1) and 80.64% of zearalenone (ZEN) from potato dextrose broth medium. Yeast cells and culture broth decreased FB1 by 34.16% and 82.59%, along with decreasing ZEN by 51.83% and 75.34%, respectively. Optimization results showed highest degradation percentage after incubation of 0.6g yeast cells for 96h at 37°C, pH 6 in the presence of glucose and peptone as C and N sources. Likewise, incubation with Mg^{2+} and Se^{2+} ions significantly increased toxin degradation. Proteinase K, EDTA and heat treatment decreased degradation activity, proving that the degradation is enzymatic. TEM observation confirmed the inhibitory effect of Yarrowia broth on F. moniliforme, indicating that inhibition of the toxin-producing strain is another mechanism of action. Conclusively, the applied yeast has detoxification capability of mycotoxins and especially FB1 due to more than one mechanism. This study spotted the light on Yarrowia lipolytica strain that can be used to safely remove ZEN or FB1 toxicity from food and animal feed.*

Keywords: *Mycotoxins, detoxification, Fusarium moniliforme, Yarrowia lipolytica and TEM.*

I. Introduction

Fusarium is a large cosmopolitan genus of imperfect fungi and it is of primarily interest because numerous species are important pathogens, produce a wide range of secondary metabolites, and/or cause opportunistic mycoses in humans and animals [1]. In recent years, mycotoxin contamination of cereal grain and animal feed with mycotoxins has become a global concern. It is estimated that it may affect as much as 25% of the world's food crops each year. Mycotoxins are toxic secondary metabolites produced by certain species of molds that commonly contaminate agricultural crops. The most common mycotoxins found in animal feed are aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone and ergot alkaloids [2].

Currently, 28 structural fumonisin analogs are known, and the most abundant analogue in nature is fumonisin B1 (FB1), followed by fumonisin B2 (FB2) and fumonisin B3 (FB3) [3&4].

Fumonisins are produced mainly by *Fusarium verticillioides*, and fumonisin contamination in corn has been observed in various areas of the world [5&6]. FB1 has been implicated in disorders in animals such as leukoencephalomalacia in horses, pulmonary oedema syndrome in pigs, and showing nephrotoxicity, hepatotoxicity, and hepatocellular carcinogenicity in rats [7&8].

The International Agency for Research on Cancer (IARC) classified FB1 as a probable human carcinogen (class 2B) [9]. To protect human health, the European Union (EU) has set a maximum level of 1000 mg/kg for the sum of FB1 and FB2 in corn and corn-based foods intended for direct human consumption [10].

Zearalenone (ZEN; 6-(10-hydroxy-6-oxo-trans-1-undecyl-β-resorcylic acid lactone) is a lactone derivative of resorcylic acid and a nonsteroidal estrogenic mycotoxin produced by several species belonging to the genus *Fusarium* [1]. High concentrations of ZEN are usually found in maize and hay stored under warm, humid conditions. However, ZEN is also a food contaminant, with concentrations as high as 289 µg/g [11&12]. The estrogenic and possible carcinogenic effects of ZEN are of further concern [13-15] causing liver and kidney damage [16], endocrine disruption [17], as well as immunotoxicity were demonstrated [18]. These findings underline the urgent need to eliminate ZEN contamination in food and food products for human consumption. Because of the economic losses engendered by the mycotoxins and their negative impact on human and animal health, many strategies for detoxifying contaminated food and animal feed have been examined, including physical, chemical, and biological detoxification methods [19]. Of these, the biological control of *Fusarium* mycotoxins is an attractive alternative for efficiently eliminating toxins and thus safe-guarding the quality of food and feed [20]. Limitations such as loss of product nutritional and sensory qualities as well as the expensive equipment required for these techniques have encouraged the recent emphasis on biological methods [21]. Biological decontamination methods are being widely studied and may be a very promising choice, provided

they show to be efficient, specific, cost-effective, and are environmentally friendly [22&23].Yeast strains, due in large part to their GRAS (Generally Regarded As Safe) status and probiotic use, are of particular interest for reducing the bioavailability of mycotoxins [24]. However, a correlation between the β-D-glucan amount in the yeast cell wall and the efficacy of sequestering ZEN, aflatoxin B1, deoxinivalenol, patulin and ochratoxin A were demonstrated [25]. On the other hand, several studies report that some fungal, bacterial and yeast strains were able to in vitro biodegrade AFB1, OTA and ZEN [23, 26-28]. This fact suggests that more than one mechanism of mycotoxin clearance could be involved. The purpose of this study was to evaluate the ability of *Yarrowia lipolytica* strain, obtained from feedstuff, to remove fumonisin and zearalenone in vitro and the correlation between the factors affecting the mycotoxin reduction and yeast strain. Also, the possible mechanism of mycotoxin detoxification was studied.

II. Materials And Methods

1.1. Tested strains

Among thirty yeast strains from our laboratory collection used in biodetoxification screening experiments; *Yarrowia lipolytica* NRRL Y-1094 strain was then selected for studying possible detoxification mechanism. *Fusarium moniliforme* Sheldon ATCC 38932 stain was used as a mycotoxin producer strain. Yeast cells were cultivated in potato dextrose broth (PDB) medium for five days at 37°C together with mycotoxin standards. Potato dextrose broth (PDB) with tested mycotoxin without yeasts as well as individual yeast strains without mycotoxins in PDB were used as positive and negative controls.

1.2. Chemicals:

FB1 and ZEA standards were purchased from Sigma Aldrich (UK) and a stock solution was prepared at a concentration of 500 µg/mL in acetonitrile-water (1:1, v/v). *O*-phthaldialdehyde (OPA), 2-mercaptoethanol, sodium dihydrogenphosphate dihydrate, sodium tetraborate, hydrochloric acid, phosphoric acid, sodium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate and potassium chloride were of analytical grade from Merck (Darmstadt, Germany). Acetonitrile and methanol were of HPLC grade.

1.3. Sample preparation:

Treated and non-treated samples infected with *Fusarium moniliforme* were extracted using the method of Sydenham *et al.* [29] with some modifications. In brief, the samples (100ml) was extracted three times with acetonitrile/methanol/water (25/25/50, v/v/v; 100 mL) for 10 min. each. After centrifugation, an aliquot of the supernatant was cleaned-up using anion extraction cartridges (Varian, Harbor City, CA, USA), which were preconditioned with methanol and water (3:1). After washing with methanol, the toxins were eluted with acetic acid-methanol (1:99, v/v; 10 mL), dried under nitrogen at 60°C and then stored at 4°C prior to analysis.

1.4. Chromatographic conditions

Dried samples were dissolved in methanol and an aliquot derivatized with o-phthaldialdehyde (OPA) for HPLC separation as previously reported [29&30]. For OPA reagent preparation, 40 mg of *O*-phthaldialdehyde was dissolved in 1 mL of methanol, and diluted with 5 mL of Na2B4O7 solution, then 50 µL of 2-mercaptoethanol was added with gentle mixing. The formed mixture was stored in the dark in a capped amber vial. The reversed-phase HPLC separation was performed on a C18 column (150×4.60 mm, Cosmosil 5 C18-AR, 5 µm). In case of FB1 detection, the column was eluted isocratically at a flow rate of 1 mL/min with methanol-0.1 M sodium dihydrogen phosphate (77:23; v/v) mobile phase adjusted to pH 3.35 with o-phosphoric acid and optimal UV detection was obtained at 335 nm. However, ZEN was separated with a mobile phase of water: acetonitrile (50:50, v/v) at a flow rate of 1 ml/min. and detection was carried out at 274 nm [31]. Injection volume was 20 µL. The HPLC instrument was configured with an Agilent (Waldbronn, Germany) 1100 series UV detector. Calibration was carried out in the range of 10-1000 µg/L. Data was collected and analyzed by Agilent ChemStation software and quantification was achieved by comparison of peak areas with those of authentic standards.

1.5. Detoxification study in submerged fermentation:

The culture medium containing the mycotoxin was inoculated with the yeast in a 150 mL flask. In parallel, non-contaminated and inoculated media (control 1) and contaminated but not inoculated media (control 2), were prepared. In all cases, triplicates were tested.Flasks containing the fermentative medium were inoculated for 240 h, at 37 °C under orbital agitation (200 rpm). A sample of fermented media (5 mL) was removed for analysis every 24 h and the residual mycotoxin was quantified. The sampling procedure was performed aseptically.

The percentage of mycotoxin degradation (D%) was calculated using the following formula:

$D\% = (C1 - C2) / C3 \times 100\%$

where:

C1: The concentration of mycotoxin in the control after fermentation;
C2: The concentration of mycotoxin in treatment after fermentation;
C3: The initial concentration of mycotoxin before fermentation.

1.6. Optimization of fermentation conditions:

All optimization experiments were conducted in PDB. 24 h-old yeast-inoculum was inoculated in PDB (100 mL) containing mycotoxin (5 µg/mL) in a 250 mL flask with four replicates for each treatment. Inoculated cultures were incubated at 37 °C with agitation at 200 rpm for 24 h in a shaker incubator. After incubation, the cells were removed by centrifugation at 10,000 *g* for 5 min. The resulting supernatant was used for mycotoxin analysis. Sterile PDB was used to substitute microbial culture in the control. The mycotoxin degradation tests were performed as described in Section 2.5.

2.6.1. Optimization of fermentation medium:

Six culture media, including potato dextrose (PDB), Sabouraud dextrose (SDB), malt extract (MEB), yeast malt extract (YMB), yeast extract potato dextrose (YPD) and corn liquor broth media were screened to select the best fermentation medium for mycotoxin detoxification.For the carbon source tests, peptone was added as the nitrogen source during the tests. 20% glucose, galactose, arabinose, sucrose, maltose, mannitol, starch, lactose, ribose, trehalose, and fructose were individually introduced into PDB to determine the effect of the carbon source on mycotoxin degradation. The effect of different concentrations of the best carbon source on mycotoxin degradation was studied in PDB with 0.5%, 1%, 2%, 3%, and 4% of optimized carbon source.
For the nitrogen source tests, the optimized carbon source was added as the carbon source. 0.5% peptone, beef extract, tryptone, sodium nitrate and ammonium chloride were individually introduced into the PDB medium to determine the effect of the nitrogen source on mycotoxin degradation. The effect of different concentrations of the best nitrogen source on mycotoxin degradation was also studied in PDB with 0.2%, 0.5%, 0.7%, and 1% of optimized nitrogen source.

2.6.2. Optimization of incubation temperature, period, amount of inoculum and pH:

To measure the effect of temperatures, the reaction mixture was incubated at 10, 20, 30, 37, and 45°C with agitation at 200 rpm for 96 h in a shaker incubator. Different incubation periods (1, 4, 12, 24, 48, 72, 96, 120, 168 and 240 h) were also tested to explore the mycotoxin detoxification kinetics.
In the pH tests, initial pH value in culture medium was adjusted to 3, 4, 5 and 6 by using HCl, and to 7, 8, 9, and 10 by using NaOH.
To test the effect of the amount of inoculum, the cell pellets were washed three times with PBS, then 0.2, 0.4, 0.6, 0.8 and 1.0 g pellets were suspended in 9 mL PBS solution infused with 1 mL, 50 µg/mLZEN or FB1, and incubated at 37 ºC, 200 rpm under aerobic conditions for 96 h, respectively.
To evaluate the effect of different metal ions, Fe^{3+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Se^{2+} and Li^{+} (in the form of $FeCl_3$, $MgSO_4$, $ZnCl_2$, $CuSO_4$, $MnCl_2$, $SeCl_2$, and LiCl) in a concentration of 5 mM was separately added to the reaction mixture. The reaction mixture was incubated at 37 °C with agitation at 200 rpm for 96 h in a shaker incubator.

2.6.3. Degradation of mycotoxin by the culture broth, cells and cell extracts:

To identify the mechanism by which *Y. lipolytica* strain exhibited detoxification activity, the cells, cell extract, and culture broth were assayed in separate experiments as described before [21, 23]. The yeast cells were separated from the broth medium by centrifugation at 10,000 *xg* for 5 min., after which the culture broth was sterilized using 0.2-µm disposable syringe filters (Millipore, Bedford, MA, USA). The yeast cells were then divided into two portions based on treatment either viable cells or autoclaved cells (121 ºC for 20 min.).The cell extracts were prepared by the following procedures; the cell pellets were resuspended in phosphate buffered saline (pH 7.4) in preparation for cell rupture. The suspension was disintegrated thrice on ice by using a Virsonic ultrasonic cell disintegrator (Virtis, USA). The disintegrated cell suspension was centrifuged at 12,000 *xg* for 20 min at 4 °C, and then the supernatant was filtered aseptically using sterile 0.2 µm cellulose pyrogen free filters.The degradation tests were carried out after incubation at 37 °C with agitation at 200 rpm for 72 h in a shaker incubator as described in 2.5. PDB was used to substitute supernatant in the control. The phosphate buffered saline was used to substitute cell and cell extracts in the control.

2.6.4. Effects of protease, heat and EDTA on zearalenone degradation activity in culture broth:

In order to study the factors affecting the degradation activity, the culture broth were treated separately with 1 mg/ml proteinase K plus 1% SDS for 1 h at 100 ºC water for 10 min and 0.1 M EDTA [33]. The effect of heat treatment was also determined by dipping the culture broth at 50 and 80 °C for 2, 4, and 6 h, respectively. The culture broth was included as negative control; however, sterile PDB media with ZEN or FB1 were included as blank control. ZEN or FB1 was added to all of the preparations described in previous sections, to a final concentration of 5 µg/mL.All of the samples were incubated at 200 rpm at 37 ºC under for 96 h.

1.7. Antifungal activity of yeast metabolites:

The antifungal activity of extracellular metabolites produced from *Yarrowia lipolytica* was investigated against *Fusarium moniliforme* using agar well diffusion method with slight modifications [34]. Briefly, *Fusarium moniliforme* culture was spread onto solidified MEA media in Petri-dish (150 mm x 20 mm). The plate was then punched with a 6 mm diameter cork-borer to create wells. The yeast metabolites were added into each well. The plates were first incubated for 2 h at 4±2 °C followed by 48 h at 28±2 °C. Then, the antimicrobial activity was determined by measuring the mean diameters of inhibition zone in millimeter.

1.8. TEM observations of treated cells:

The treated and untreated *Fusarium moniliforme* cells were observed under Transmission Electron Microscope (TEM). The samples were prepared by standard protocol. Samples were fixed in 1% Glutaraldyhde then washed in 0.1 M buffer, 1% Osmium tetroxide was used for post-fixation and again washed with 0.1 M buffer. The samples were dehydrated in a series of ethanol and acetone, infiltrated, embedded in epoxy EMbed 812 resin and sectioned using Leica Ultracut ultramicrotome. These ultra-thin sections (60 nm) were cut and placed on copper grids, counterstained with saturated uranyl acetate and aqueous lead citrate. Finally, the grids were dried in a desiccator and examined at 80 kV using TEM (JEOL-JEM 1010, Japan) at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, to study the action of yeast metabolites on fungal cells and any morphological changes.

1.9. Statistical Analysis:

All specific experiments were repeated three times. Statistical analysis was performed using Statistix 8.1 (Analytical Software, Tallahassee, FL, USA, 2005).

III. Results And Discussion

1.10. Degradation of FB1 and ZEN by the culture broth, cells and cell extracts:

With the growing frequency of food and feed safety issues, there has been an increased focus, specifically, on bio-pollution problems, in which mycotoxins are one of the main factors causing food and feed contamination. In this study, thirty yeast strains were screened for biological detoxification screening capabilities; the screening experiments showed that *Yarrowia lipolytica* NRRL Y-1094 strain exhibited the highest ability to reduce mycotoxins produced by *Fusarium moniliforme* Sheldon ATCC 38932.

The FB1 and ZEN degradation activity of *Y. lipolytica* culture broth was significantly stronger than cells and cell extracts **(Fig. 1)**. Moreover, the dead cells (autoclaved) exhibited the lowest detoxification activity against the two mycotoxins (**Fig. 1**). The culture broth, viable cells, autoclaved cells and cell extracts of *Y. lipolytica* could degrade 82.59%, 34.16%, 13.82% and 25.89% FB1, as well as 75.34%, 51.83%, 20.29% and 18.41% ZEN, respectively. Some similar results were reported. Culture supernatant of *Stenotrophomonas maltophilia* was able to degrade 78.7% mycotoxin after 72 h incubation [35]. The extracellular extracts of *Rhodococcus erythropolis* liquid culture could degrade 66.8% aflatoxin B1 after 72 h incubation [36]. In addition, the active ingredient in the culture supernatant was considered to be enzymatic [36].

1.11. Optimization of fermentation conditions for FB1 and ZEN degradation:

In this study, *Yarrowia lipolytica* could remove 84.92% of fuminosin B and 80.64% of zearalenone from potato dextrose broth medium. Lower detoxification percentages were detected by the other tested culture media (**Fig. 2**). These detoxification levels were increased with optimization of the fermentation conditions.

The effect of different incubation times on the FB1 and ZEN degradation activity of *Y. lipolytica* was also tested. The degradation process by *Y. lipolytica* was relatively fast and continuous, with 9 and 15.4 % of FB1 and ZEN removed in the first 1 h, 43.9 and 36.8% removed after 12 h, 54.6 and 44.5%removed after 24 h, 70.8 and 62.9% removed after 48 h, 82.6 and 75.3% removed after 72 h, 90.76 and 84.2% removed after 96 h (**Fig. 3**). Non-significant difference in the degradation of FB1 and ZEN could be detected after further increase in incubation time from 96 to 240 h. Alberts *et al.* reported that a significant ($p < 0.05$) reduction in aflatoxin B1 was already observed after 2h in the presence of *Rhodococcus erythropolis* extracellular extracts with only 33.2% residual aflatoxin B1 after 72 h [36]. Additionally, Hormisch *et al.* found that the aflatoxin B1 concentration was reduced to amounts of 70% to 80% of the initial concentration within 36 h by *Mycobacterium fluoranthenivorans* [37].

When using carbon sources like glucose, galactose, arabinose, sucrose, maltose, mannitol, starch, lactose, ribose, trehalose, and fructose, the degradation in the presence of glucose was significantly higher than other carbon sources **(Fig. 4)**. Moreover, the detoxification process increased with the increased concentration of glucose. Among the five different concentrations of glucose tested, 4% was the best for ZEN degradation; however, 3% was the best for FB1 degradation **(Fig. 5)**. Different carbon sources affect the growth of microorganisms. The growth of microorganisms was stimulated when the carbon source was utilizable [38].

When the carbon source was difficult to be utilized, the microorganism strains tended to utilize other carbon sources, which might promote a decrease in other carbon-based compounds [39,40].As shown in **Fig. 6**, when the nitrogen source was tested, peptone exhibited significant higher effect on the detoxification process compared with the other N sources (beef extract, tryptone, ammonium salt and sodium nitrate). Among the four different concentrations of peptone tested, 0.5% was the most suitable for both mycotoxins degradation **(Fig. 7)**. Appropriate nitrogen source and concentration would not only stimulate the growth but also affect the expression of biosynthetic genes and therefore the production of microorganisms [40-42].

The effect of temperature on FB1 and ZEN degradation by the culture broth of *Y. lipolytica* is shown in **Fig. 8**. The highest percentage of degradation was obtained at 37 °C. The temperature affects the growth of microorganisms, thus the metabolic production. The initial pH value in PDB showed a significant effect on FB1 and ZEN degradation **(Fig. 9)**. In the present study, the highest percentage of degradation was obtained at pH 6 and lowest at pH 3 and 10. The pH of medium is a very important environmental factor, which is often neglected. Many investigators claimed that the different morphology of fungal mycelia under different initial pH values was the critical factor in biomass accumulation and metabolite formation [43,44]. The correlation of FB1 and ZEN degradation with pH values is typical for enzymatic reactions.

The effect of amount of inoculum on FB1 and ZEN degradation was also analyzed. The optimal fermentation conditions observed with the amount of inoculum of 0.6g, inoculated cultures when incubated at 37 °C for 96 h in a shaker incubator **(Fig. 10)**. It has been reported previously that growth and enzyme production of microbes were affected by variations in incubation temperature, period and amount of inoculum [21,45].

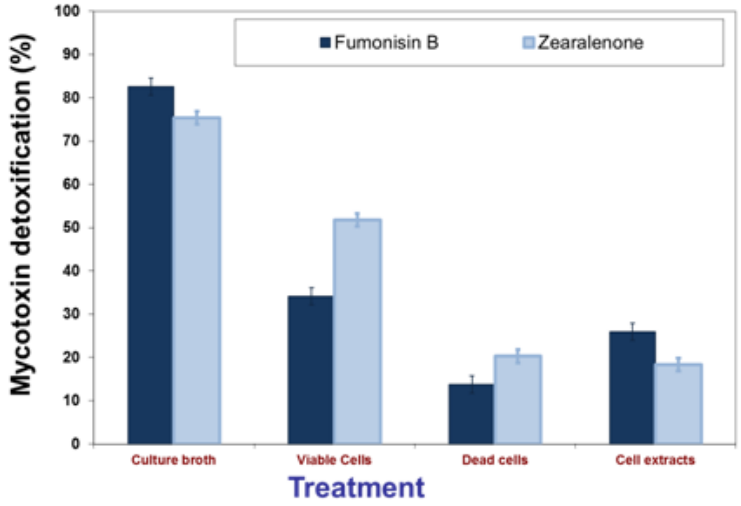

Figure1. Detoxification of the mycotoxin by the culture broth, cells and cell extracts of *Yarrowia lipolytica* after 72 h incubation. The medium was used to substitute culture broth in the control. The phosphate buffered saline was used to substitute cells and cell extracts in the control. Results are expressed as means ± SEM from three separate experiments.

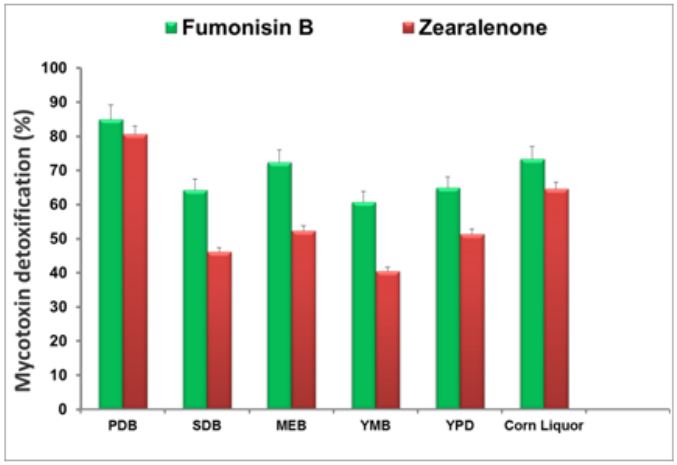

Figure 2. Effect of different culture media on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

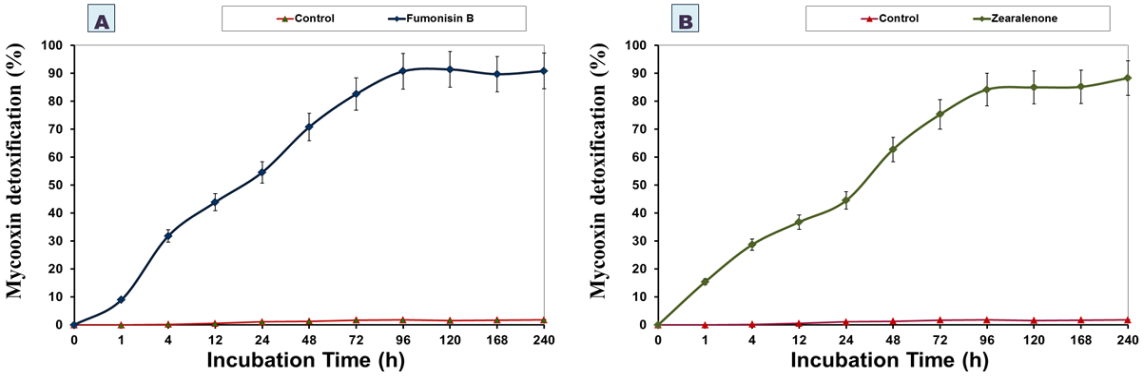

Figure 3. Kinetics of mycotoxin detoxification during 240 h of fermentation by *Yarrowia lipolytica* showing the effect of different incubation periods on the detoxification process of fumonisin B (A) and zearalenone (B).

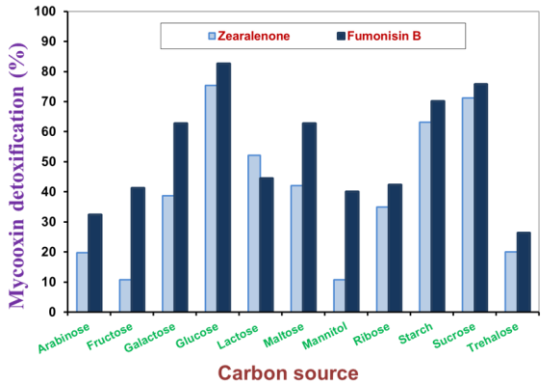

Figure 4. Effect of different carbon sources on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

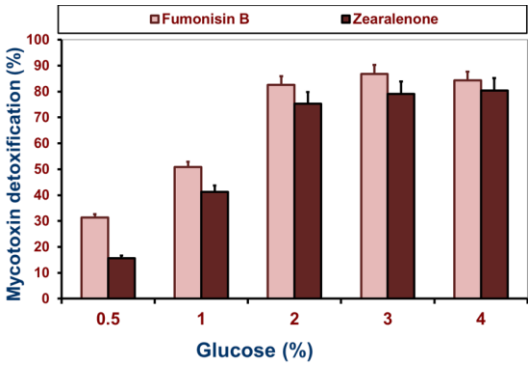

Figure 5. Effect of different glucose contents on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

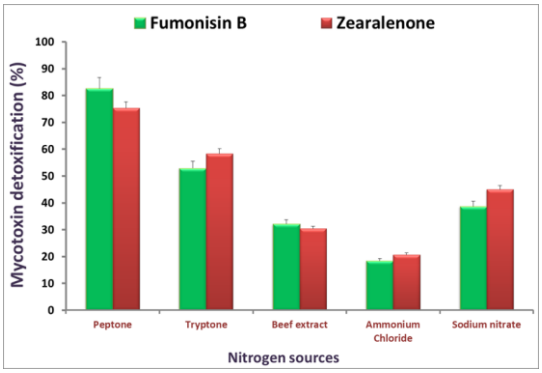

Figure 6. Effect of different nitrogen sources on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

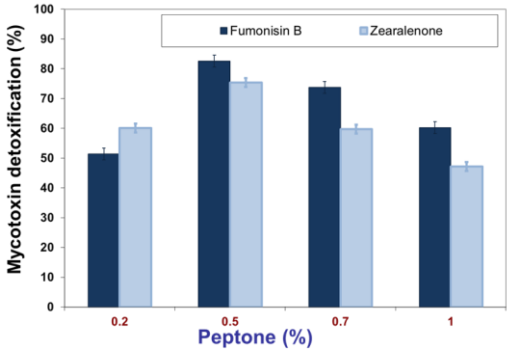

Figure 7. Effect of different peptone contents on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

The effect of different metal ions on FB1 and ZEN degradation is shown in **Fig. 11**. Compared to the treatment without ions, Li^{+} and Zn^{2+} showed the strongest inhibition function ($p < 0.001$) in mycotoxin detoxification process. Likewise, incubation with Se^{2+} and Mg^{2+} significantly increased toxin degradation. Fe^{3+}, Cu^{2+} & Mn^{2+} differed in their effect **(Fig. 11)**. The effects of metal ions on degradation activity further supported the enzyme involvement in mycotoxin degradation by the isolate. Also, the correlation of mycotoxin degradation with pH values is typical for enzymatic reactions. Similar results were reported that Zn^{2+}, Cu^{2+}, and Mn^{2+} inhibited aflatoxin B1 degradation by *Flavobacterium aurantiacum* [46, 47].

1.12. Possible mechanism of FB1 and ZEN detoxification:

However, enzymatic effects in culture broth were suggested as the possible mechanism by which significantly degraded the mycotoxin more than 80%. The percentage of mycotoxin degradation decreased when the culture broth dipped at 50 and 80 °C for 6 h **(Fig. 12)**. Heat treatment decreased the FB1 and ZEN degradation activities of yeast culture broth. The higher the temperature was, the faster the activity decreased. Similarly, Guan *et al.* reported that when culture supernatant was heated (boiling water bath for 10 min), no mycotoxin degradation activity was observed [35].

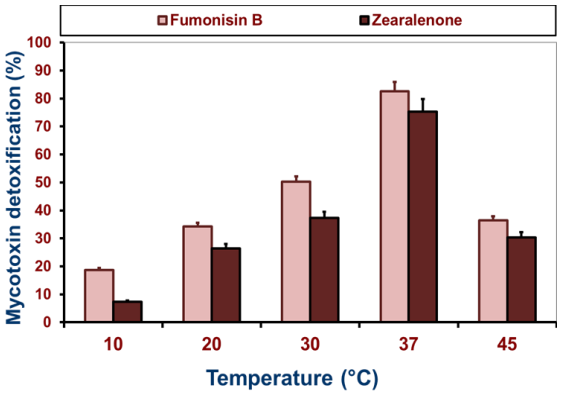

Figure 8. Effect of different temperature on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

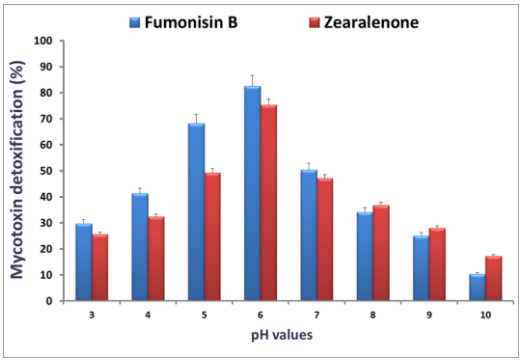

Figure 9. Effect of different pH values on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

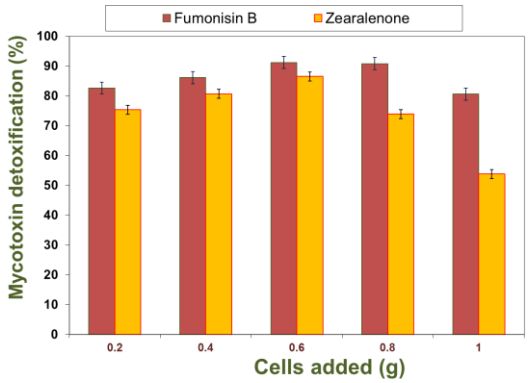

Figure 10. Effect of cell amounts on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

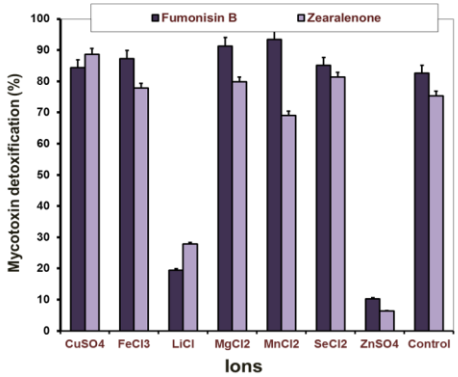

Figure 11. Effect of metal ions on the mycotoxin detoxification by *Yarrowia lipolytica* after 96 h incubation.

After treatment with proteinase K and heat **(Fig. 12&13)**, the pooled culture broth retained low mycotoxin degradation activity, which indicated that enzymes from the pooled active culture broth are involved in the degradation. EDTA, as metal chelating agent, can destroy the FB1 and ZEN degradation activity **(Fig. 13)**, which indicated that some metal cations are required for the enzymes in the active fractions to degrade FB1 and ZEN as previously reported [47]. All indications are that an extracellular metalloenzyme is responsible for the degradation by culture broth. Moreover, incubation of the toxin with either heat-treated or viable cells resulted in lower percentage of ZEN removal from the medium, indicating that binding, not metabolism, was the removal mechanism by the cells. The reason is that both adsorption and degradation effects exist in the yeast suspension.

The antifungal activity of the culture broth produced from *Yarrowia lipolytica* was observed against *Fusarium moniliforme* using agar well diffusion method **(Fig. 14)**. Transmission electron microscopic study confirmed the inhibitory effect of *Yarrowia ipolytica* broth on *Fusarium moniliforme*, and indicated that the mycelium had lost its cytoplasmic contents and became highly vacuolated compared to untreated mycelia **(Fig. 15)**. Similar reports [11, 28] showing the inhibition of toxin and toxin-producing strains are in the same line of the current study. Previously, Molnar described a new yeast strain, *Trichosporon mycotoxinivorans*, which is able to degrade ZEN to carbon oxide and other non-toxic metabolites [48]. Takahashi-Ando characterized a novel lactonohydolase enzyme from the fungus *Clonostachys rosea* which convert ZEN to a less toxic metabolite [26]. Several *Rhizopus* strains, including *R. stolonifer*, *R. oryzae* and *R. microsporus*, were found to completely degrade ZEN [49]. Similarly, *Rhodococcus pyridinivorans*, *Bacillus*, and *Lactobacillus* strains were proved to be very efficient to eliminate ZEN in culture media [50-52].

Products containing yeast materials have potential to adsorb mycotoxins due to the physical properties of the yeast cell wall, which has structures that allow for binding of mycotoxins [53,54]. The β-glucan of yeast can from single or triple helix polysaccharide chains which can give adsorptive capacities for mycotoxins through hydrogen and ionic bonding, and van der Waals interactions [55]. Previous *in vitro* studies have shown that β-glucan isolated from yeast cell walls has the capacity to adsorb up to 50% of ZEA [56]. The yeast fermentation product may also play a role in adsorbing mycotoxins, due to the fact that it contains β-glucan. Together, these effects of the yeast fermentation product may improve the ability of the animal to fight mycotoxins [24, 57].

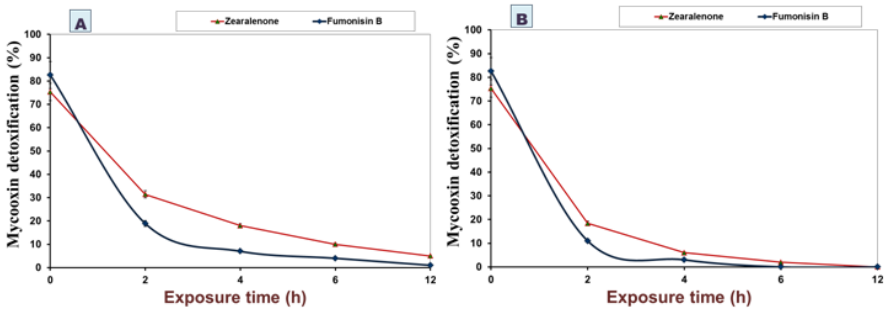

Figure 12. Effect of heat treatment on mycotoxin detoxification during 12 h of fermentation by Y. lipolytica showing the effect of incubation at 50 °C (A) and 80 °C (B) on the detoxification process.

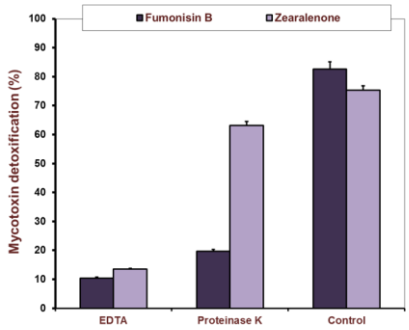

Figure 13. Effect of proteinase K and EDTA treatment on mycotoxin detoxification on the detoxification process.

Figure 14. The inhibitory effect of *Yarrowia lipolytica* extracellular metabolites (broth) on growth of *Fusarium moniliforme* showing the inhibition zones in response to yeast metabolites (1) and no inhibitory effect on cell extract (2).

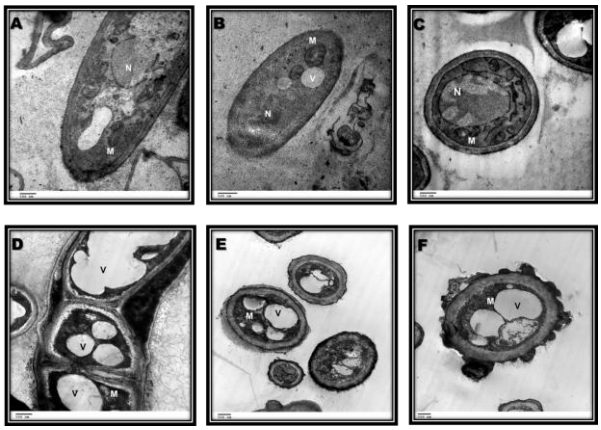

Figure 15. Transmission Electron Micrographs representing morphological changes in *Fusarium moniliforme* treated with *Yarrowia lipolytica*. ***A-C:*** *Fusarium moniliforme* control showing normal mycelium and conidium. ***D-F:*** *Fusarium moniliforme* treated with *Y. lipolytica* extracellular metabolites (broth) showing highly vacuolated mycelium. (where, M: Mitochondria, N: nucleus, V: vacuole); scale bar = 500nm.

IV. Conclusions

In this study, the environmentally friendly *Yarrowia lipolytica* NRRL Y-1094 strain reduces FB1 and ZEN contamination as well, via mechanisms involving binding and metabolism of the toxin along with inhibitory effect on the toxin-producing strain *Fusarium moniliforme*, thereby alleviating hazards to human and animal health. In the culture broth, the activities of extracellular enzymes were most likely responsible for FB1 and ZEN degradation. This ability to remove the toxin was greater in yeast exponential growth phase, and that the process was a quick one, being saturated after 1 hour of contact. The great advantage in the commercial use of this yeast as detoxification agent is that it is already used in a wide range of fermented food products, being recognized as safe. Therefore, we suggest that *Yarrowia lipolytica* NRRL Y-1094 strain can be used to safely remove ZEN or FB1 toxicity from food and animal feed. Functional and technological tests should be performed to validate *in vivo* efficiency.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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