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Efficiency of Different Sources of *Saccharomyces cerevisiae* to Bind Aflatoxin B_1 in Phosphate Buffer Saline

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Abstract

Aflatoxins, a group of carcinogenic mycotoxins, can cause acute and chronic intoxications and also liver cancer in humans and animals. Aflatoxin B_1 (AFB_1) is the most potent, having proven toxic properties. Biological decontamination of mycotoxins is one of the well-known strategies for management of mycotoxins in foods and feeds, presenting some advantages over physical and chemical methods. Among the different possible decontaminating microorganisms, *Saccharomyces cerevisiae* is a potential group since it is widely used in preservation and food fermentation. *Saccharomyces cerevisiae* cell wall consists of a network of β-1,3 glucan back bone with β-1,6 glucan side chains, which is attached to highly glycosylated mannoproteins making the external layer. Binding of different mycotoxins to yeast cell surface has been reported. This study was carried out to investigate the efficiency of *S. cerevisiae* to remove AFB_1 in Phosphate Buffered Saline (PBS) solution (pH 7.3 25°C). *Saccharomyces cerevisiae* concentration from four different sources (dried yeast of sugar cane, autolyzed yeast, cell wall and brewery dehydrated residue) was determined by a Neubauer-counting chamber, using 1x1010 non-viable cells for each 3.0 mL of PBS containing 0.5μg L^{-1} AFB_1. The assay was performed at contact times of 5, 10, 20 and 30 minutes. Among all analyzed yeasts, the dried yeast of sugar cane presented highest removal capacity of AFB_1, with an average reduction of 98.3%. Autolyzed yeast and brewery dehydrated residue presented extensive removal capacity, with averages of 93.8 and 84.6%. The yeast cell wall showed the lowest removal capacity (82%).

Keywords: AFB_1; *S. cerevisiae*; Decontamination; Binding; PBS

Introduction

Aflatoxins are one of the most important mycotoxins known, being *Aspergillus* species distributed worldwide, although their optimal growth conditions are relative humidity of 80-85% and temperature around 30°C. Aflatoxins are secondary metabolites of low molecular weight produced by filamentous fungi, particularly *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, distinguished by their wide distribution in food and pronounced toxic properties [1]. There are currently 18 similar compounds described by the term aflatoxin, but the most prevalent and toxic is aflatoxin B_1 (AFB_1) [2]. Aflatoxins are also of great importance for the Public Health, as they are main factors involved in the etiology of human hepatic cancer, as a consequence of the ingestion of contaminated foods.

After oral ingestion, AFB_1 is efficiently absorbed and bio transformed before urinary and fecal excretion. Enzymes of the cytochrome P_{450} family are responsible for the biotransformation of absorbed aflatoxins. These enzymes convert AFB_1 into its carcinogenic form, AFB-8,9-epoxide, which bonds covalently to DNA and serum albumin, producing AFB_1-N_7-guanine and lysine adducts, respectively. The bond between AFB_1 and DNA modifies the structure and biological activity of DNA, leading to the basic mutagenic and carcinogenic mechanisms of the toxin. Besides being epoxided, AFB_1 can be also oxidized into several other derivatives. The main hydroxylated metabolites are aflatoxin M1 ($AFBM_1$), aflatoxin Q_1 (AFQ_1), a demethylated metabolite, aflatoxin P_1 (AFP_1), and a reduced metabolite, aflatoxicol. The most important effect of aflatoxins on human health is the Hepatocellular Carcinoma (HCC). This disease represents more than 80% of primary malignant tumors of the liver, and it is the 7th to 9th most common type of cancer worldwide affecting men and women, respectively [3].

The best way to prevent aflatoxin contamination is the adoption of improved agricultural practices and control of storage conditions of products. However, practical difficulties to effectively prevent contamination, along with the stability of aflatoxins under normal food processing conditions, have led to investigation on decontamination methods for food products [4]. The use of microorganisms offers an attractive alternative for the control or elimination of aflatoxins in foodstuffs [5,6]. *Saccharomyces cerevisiae* (SC) is the most effective for binding AFB_1 [7], although several Lactic Acid Bacteria (LAB) strains have shown different capabilities for binding AFB_1 in phosphate buffer solutions and in milk [8,9]. Therefore, the aim of the present study was to evaluate the ability of a SC strain from four different sources (dried yeast - DY, autolyzed yeast - AY, cell wall - CW and brewery dehydrated residue - BDR), to bind AFB_1 in Phosphate Buffer Saline (PBS) spiked with 0.5 ng mL^{-1} AFB_1, during contact times of 5, 10, 20 and 30 minutes.

Materials and Methods

SC sources

Commercially available sugar cane yeast (dried yeast from sugar cane - DY, autolyzed yeast from sugar cane - AY, cell wall from sugar

cane – CW) and *brewery yeast* (brewery dehydrated residue - BDR) were used in the experiment.

The number of yeast cells in the products was determined by light microscopy using a modified Neubauer chamber. The products were weighed to reaching a cell concentration of $1.0X10^{10}$ cells mL^{-1}. All SC cells were heat-killed, being inactivated by autoclaving at 121°C for 10 minutes before the binding assays, to avoid any possible fermentation during the contact time.

Aflatoxin B1 binding assays

AFB_1 standard solution (Sigma-Aldrich, St. Louis, USA) was diluted in acetonitrile and spectrophotometrically calibrated [10] in order to obtain a 2.5 μg mL^{-1} stock solution. A 0.15 μg mL^{-1} working solution was prepared in PBS (Laborclin Ltd., Pinhais, Brazil), pH 7.3, evaporating the acetonitrile by nitrogen injection and heating in a hot-water bath (45°C) until visible acetonitrile droplets disappeared.

The assay of AFB_1 binding in PBS was performed in triplicate as described by Bovo et al. [9] with some modifications. A volume of SC strains from each different source corresponding to $1.0X10^{10}$ cells mL^{-1} were transferred to Eppendorf tubes and suspended in 3.0 mL of PBS spiked with 0.5 ng mL^{-1} of AFB_1. Following the contact times of 5, 10, 20 and 30 min., the tubes were centrifuged at 1,800 g for 15 minutes, and the supernatant removed for analysis of AFB_1. The same procedures as described above were performed in triplicate positive controls (only spiked PBS containing 0.5 ng $mL^{-1}AFB_1$), negative controls (only different sources of SC) and non-spiked PBS controls.

Analysis of aflatoxin B_1 in PBS

Quantification of AFB_1 in PBS solutions was performed by injection of supernatant in a High-Performance Liquid Chromatograph (HPLC) Shimadzu® system (Tokyo, Japan), consisting of a fluorescence detector RF-10A XL (Shimadzu®) equipped with a Synergy Fusion column 4μm C18 4.6×150 mm (Phenomenex®, Torrance, USA) and autosampler SIL- 10AF (Shimadzu®). A flow rate of 1 mL/min was used with a mobile phase containing water, acetonitrile, and methanol (60:20:20). Detection was made at an excitation wavelength of 366 nm and emission at 428 nm. Detection limit for AFB_1 was 0.01 ng/mL, as considered by the minimum amount of AFB_1 that could generate a chromatographic peak three times over the baseline standard deviation. Under these conditions, the retention time of AFB_1 was 6.9 min.

The equation below was used to determine the percentage of AFB_1 bound by the microorganisms tested in each assay. Letters B, C, D and E are the mean areas of chromatographic peaks of positive controls, non-spiked PBS controls, sample analyzed and negative controls, respectively.

$$A = \left\{ \frac{[(B-C)-(D-E)]}{B-C} \right\} \times 100$$Statistical analysis

Statistical analysis of AFB_1 binding assays was carried out in the General Linear Model of SAS® [11] by using the Tukey Test for significant differences between the sources tested (DY, AY, CW and BDR) and contact time (5, 10, 20 and 30 min.) at $p<0.05$.

Results and Discussion

The results show the AFB_1 levels at PBS in the binding assays with heat-killed SC cells from four different sources. AFB_1 levels in spiked PBS (0.5 ng AFB_1 mL^{-1}) treated with inactivated dried yeast from sugar cane (DY) cells (1010 cells mL-1) for 5, 10, 20 and 30 min were < 0.010 ng mL^{-1}, 0.021 ± 0.003 ng mL^{-1}, 0.022 ± 0.005 ng mL^{-1} and 0.035 ± 0.002 ng mL^{-1}, respectively. The treatment DY achieved the best results in the binding assays, but without statistically significant differences on the contact time.

The second best response was achieved using the treatment of autolyzed yeast from sugar cane (AY). The values of AFB1 in PBS for AY treatment were 0.046 ± 0.008 ng mL^{-1}, 0.025 ± 0.006 ng mL^{-1}, 0.034 ± 0.003 ng mL^{-1} and 0.096 ± 0.005 ng mL^{-1} for 5, 10, 20 and 30 min, respectively.

The mean percentages of AFB_1 bound by the CW in PBS were 80.1 ± 0.5%, 78.3 ± 0.9%, 83.6 ± 0.7% and 86.1 ± 0.8% for 5, 10, 20 and 30 min, respectively. Compared to the CW, BDR cells had higher capability to bind AFB_1 in PBS (81.0 ± 0.3%, 86.0 ± 0.8%, 83.7 ± 0.2% and 87.8 ± 0.7% for 5, 10, 20 and 30 min, respectively), although there were no significant differences between the treatments and contact times evaluated.

Compared to the CW and BDR treatments, DY and AY had higher ($p<0.05$) capability to bind AFB_1 in PBS, although there were no differences ($p>0.05$) between the contact times evaluated. When comparing DY with AY, a non-significant increase ($p>0.05$) was observed in the percentages of AFB_1 bound, which mean values ranged from 97.9 ± 0.5% to 94.9 ± 1.3% (Table 1).

***S. cerevisae* products**	**% of bound AFB_1 (mean ± SD)**			
	5 min.	**10 min.**	**20 min.**	**30 min.**
Dried yeast	99.3 ± 0.2^{a}	97.9 ± 0.5^{a}	97.8 ± 0.8^{a}	96.5 ± 1.1^{a}
Autolyzed yeast	95.3 ± 1.4^{a}	97.5 ± 1.1^{a}	96.6 ± 1.6^{a}	94.9 ± 1.3^{a}
Cell wall	80.1 ± 0.5^{b}	78.3 ± 0.9^{b}	83.6 ± 0.7^{b}	86.1 ± 0.8^{b}
Brewery residue	81.0 ± 0.3^{b}	86.0 ± 0.8^{b}	83.7 ± 0.2^{b}	87.8 ± 0.7^{b}

Table 1: Percentages of aflatoxin B_1 bound to *S. cerevisae* products at different contact times in PBS ($^{a-b}$ In the same column, means followed by different superscript letters differ significantly ($p<0.05$)).

By the findings of this study, it is apparent that celular viability is not a prerequisite for removal of AFB_1 by SC. The mechanism involved in SC ability to bind aflatoxins remains unclear. It is currently accepted that yeast cell wall has the ability to adsorb the toxin [12-14]. Bueno et al. [12] and Lee et al. [8] concluded that both viable and non-viable SC cells have the same adsorbent ability to bind AFB_1, which is in accordance with data on removal of AFB_1 due SC in PBS as reported in the present study.

Previous reports on the use of SC for decontamination rates of AFB1 from feeds reached to 90% [15,16]. In the present study, SC cells bound from 99.3 ± 0.2% (using DY for 5 min) to 78.3 ± 0.9% (using CW for 10 min) of AFB_1 content in PBS.

When heat-killed cells of SC from different sources were used the removal efficiency of AFB_1 was highly effective (99.3 ± 0.2%). There are no previous studies evaluating the different sources of SC from co-products of alcoholic fermentation for aflatoxin removal. However, the increase in the binding percentages may be explained due the presence of a greater number of cells available for the sequestration of AFB_1.

Our results indicate that non-viable cells of SC from different sources may be used for highly removing AFB_1 from phosphate buffer saline containing up to 0.5 ng mL^{-1}. Thus further studies are necessary to investigate these effects on other matrices, such as feed, milk or cereals.

Conclusion

Heat-killed cells of different sources of SC, from co-products of alcoholic fermentation, has high efficiency (>90%) to bind AFB_1 in PBS in a relatively short period, as there were no differences in the toxin binding between the contact times of 5, 10, 20 or 30 min. Therefore the methods of aflatoxin removal employing SC have a potential application for reducing the levels of AFB_1. However, additional studies are needed to investigate the mechanisms involved in the removal process of toxin due SC and the factors that affect the stability of the toxin sequestration aiming the commercial application in the food industry.

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