In vitro ochratoxin A adsorption by commercial yeast cell walls*

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ABSTRACT. Pereyra C.M., Cavaglieri L.R., Keller K.M., Chiacchiera, S.M., Rosa C.A.R. & Dalcero A.M. ***In vitro* ochratoxin A adsorption by commercial yeast cell walls**. [Adsorção *in vitro* de ocratoxina A por paredes celulares de levedura comercial]. *Revista Brasileira de Medicina Veterinária, 37(0):00-00*, 2015. Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, (5800) Río Cuarto, Córdoba, Argentina. E-mail: lcavaglieri@exa.unrc.edu.ar

The aim of the present study was to evaluate the ochratoxin A (OTA) adsorption capacity by two kinds of commercial yeast cell walls (YCW1 and YCW2) used as dietary supplements. An *in vitro* test was designed to mimic the temperature (37ºC), pH (2) and passage time (30 min) through the stomach of a monogastric animal. The test was performed using different concentrations of YWC (100 and 200 µg/mL) and OTA (10; 80; 160 and 1000 ng/mL) and extracts were quantified by HPLC. For each OTA concentration, two independent trials varying the concentration of each YCW were performed. The two YCW assayed containing different percentages of polysaccharides, were able to adsorb similar amounts of OTA. Furthermore, the relationship mannans/β-glucans does not influence the rate of adsorption of OTA. In general, it was observed that the adsorption capacity increased with decreasing OTA concentration. Results from this work related to adsorption capacity of OTA with YCW allow predicting that other factor than 3D-structure and β-glucans and mannans could be involved. Future studies could be conducted to test the *in vivo* binding ability to alleviate the toxic effects of OTA under field conditions. Both YCW are a promising mycotoxin adsorbent to be used in animal feed to prevent mycotoxicoses.

KEY WORDS. Adsorption, mycotoxins, *Saccharomyces cerevisiae*.

RESUMO. O objetivo do presente estudo foi avaliar a capacidade de adsorção de ocratoxina A (OTA) por duas paredes celulares de leveduras comerciais (PCL1 e PCL2) usadas como aditivos alimentares. Os ensaios *in vitro* foram desenvolvidos de modo a simular as condições de temperatura (37°C), pH (2) e o tempo de passagem (30 min.) do alimento pelo estômago de um animal monogástrico. Os ensaios foram executados usando diferentes concentrações das PCL (100 e 200 µg/mL) e OTA (10; 80; 160; e 1000 ng/mL) e os extratos foram quantificados por CLAE. Para cada concentração

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de OTA, dois ensaios independentes foram realizados variando-se a concentração de cada PCL. As duas PCL analisadas que continham diferentes percentuais de polissacarídeos foram capazes de adsorver similares quantidades de OTA. Além disso, a relação mananos/β-glucanos não influenciou na taxa de adsorção de OTA. Em geral, foi observado que a capacidade de adsorção aumentava com o decréscimo das concentrações de OTA. Os resultados deste trabalho sobre capacidade de adsorção de OTA por PCL permitem supor que algum outro fator possa estar envolvido além da estrutura 3D e dos β-glucanos e mananos. Estudos futuros podem ser desenvolvidos para testar *in vivo* esta capacidade adsortiva e então reduzir os efeitos tóxicos da OTA em desafios naturais a campo. Ambas as PCL são promissores adsorventes desta micotoxina podendo ser usadas na alimentação animal para prevenir esta micotoxicose.

PALAVRAS-CHAVE. Adsorção, micotoxinas, *Saccharomyces cerevisiae*.

INTRODUCTION

The contamination of animal feed with mycotoxins represents a worldwide problem. Feed containing mycotoxin can cause serious diseases in farm animals resulting in suffering and even death and thus can cause substantial economic losses (Huwig et al. 2001). Ochratoxin A one of the most important mycotoxins is produced by some *Aspergillus* and *Penicillium* storage species (Varga et al. 2003). Nephrotoxic and carcinogenic effects on animal health have been studied in detail for many years. There are now evidences of hepatotoxic, teratogenic, mutagenic and immunosuppressive properties of OTA (Petzinger & Ziegler 2000, Walker 2002). Ochratoxin A is considered to be a genotoxic agent *in vivo* and *in vitro* in some mammalian species (WHO 2002).

Physical, chemical, physicochemical and (micro) biological approaches have been developed to reduce the impact of mycotoxins (Varga & Toth 2004). One of the most effective for controlling mycotoxin hazards in animal husbandry is based on the use of specific materials that adsorb mycotoxins, which can be used to reduce the bioavailability of mycotoxins in the digestive tract and alleviate their adverse effects on animals. Basic ingredients and dietary supplements of an animal diet may have functional proprieties in the diet. Among several studied dietary supplements yeasts mainly, *Saccharomyces cerevisiae* has shown satisfactory results when added to feed as active cells or cell wall components (Shetty & Jespersen 2006). Two major polysaccharides of *S. cerevisiae* that constitute up to 90% of the cell wall dry weight are α-D-mannan and β-D-glucan, which have remarkable properties to interact with the host immune system (Kogan & Kocher 2007). Besides its excellent nutritional value, yeasts produce a high quantity of biomass that is used in a large variety of industrial processes. The presence of macromolecules in their cell wall such as mannoproteins and β-glucans constitutes as a good source of adsorbent (Shetty & Jespersen 2006). The ability of the *S. cerevisiae* cell wall to bind zearalenone has been reported recently (Yiannikouris et al. 2003, 2004a, 2004b). However, there is little information on the adsorption of OTA by yeast cell wall. Ringot et al. (2007) examined the *in vitro* adsorption of OTA onto three yeast industry by-products. The purpose of the present study was to evaluate the OTA adsorption capacity by two kinds of commercial YCW used as dietary supplements.

MATERIALS AND METHODS

Yeast cell walls used as adsorbent and reagents

Two kinds of YCW were studied for OTA binding ability: yeast cell wall 1 (YCW1) and yeast cell wall 2 (YCW2). Their compositions are described in Table 1. Two different concentrations (100 and 200 µg/mL) of both YCW were obtained. Yeast cell wall was resuspended in solution at pH 2 (50 mL of potassium chloride 0.2 M and 13 mL of hydrochloric acid 0.2 M) for the subsequent use in the adsorption test. The pH was confirmed using a pHmeter (Model 250A, Orion Research Inc. Boston, MA 02129 USA) and the corresponding pH was adjusted using hydrochloric acid 0.2 M or sodium hydroxide 0.1 M solutions. Ochratoxin A was supplied by Sigma Chemical Company (St. Louis, Missouri, USA). It was resuspended in methanol resulting in a stock solution from which the work solution was obtained. Finally, dilutions were made with methanol to obtain five different concentrations of OTA (10; 80; 160 and 1000 ng/mL). All solvents used for chromatography were of HPLC grade.

In order to assess the OTA adsorption of each YCW,

Table 1. Composition of the main carbohydrates present in commercial yeast cell walls.

Ingredients	Composition (%)[a]	
	YWC1[b]	YWC2[c]
β-glucans	17.4	23.0
Mannans	5.9	21.0
Mannans/ β-glucans	0.34	0.91
Total of polysaccharides	23.3	44.0

[a] Commercial yeast cell wall.
[b] YCW1: yeast cell wall 1
[c] YCW2: yeast cell wall 2

an *in vitro* test was designed to mimic the temperature (37ºC), pH (2) and passage time (30 min) through the stomach of a monogastric animal was applied. The different OTA concentrations used in this assay are those naturally found in feedstuffs intended for animal production (Dalcero et al. 2002, Pereyra et al. 2009, Rosa et al. 2009).

***In vitro* adsorption capacity**

The test was performed using two concentrations of 100 and 200 µg/mL at pH 2. An aliquot of 500 µL of each YCW concentration was added to each microtube containing 500 µL of 10, 80, 160 and 1000 ng/mL of OTA, respectively. Each microtube was introduced in a centrifuge Labor 2K15 centrifuge (Sigma) at 37°C with mechanical agitation for 30 min and then centrifuged for 10 min at 14.000 rpm. The supernatant fraction was then for free toxin analysis and it was evaporated to dryness under gentle stream of nitrogen gas. Each test was performed in duplicate and controls were performed for OTA and each adsorbent. Ochratoxin A extracts were detected and quantified by high-pressure liquid chromatography (HPLC). The amount of bound toxin was calculated by subtracting the amount of free toxin found in the experimental tubes from the amount found in control tubes with no adsorbent.

Detection and quantification of OTA

The HPLC apparatus was a Hewlett Packard chromatograph with a loop of 20 µL, equipped with a spectrofluorescence detector (excitation λ 330 nm; emission λ 460 nm) and a C18 column (Supelcosil LC-ABZ, Supelco; 150 mm, 4.6 mm, 5 µm particle size) connected to a precolumn (Supelguard LC-ABZ, Supelco; 20 mm, 4.6 mm, 5 µm particle size). The mobile phase was pumped at 1.0 mL/min and consisted of an isocratic system as follows: 57% acetonitrile, 41% water and 2% acetic acid. Ochratoxin A was quantified on the basis of HPLC fluorometric response compared with OTA standard (Sigma-Aldrich, St. Louis, MO, USA; purity >99%). The detection limit of the used method was 0.1 ng/mL.

RESULTS

The ability of both yeast cell walls to bind OTA *in vitro* at pH 2 is summarized in Table 2. For each OTA concentration, two independent trials varying the concentration of each YCW were performed. In general, it was observed that the adsorption capacity increased with decreasing OTA concentration. Both YCW were able to adsorb the four studied OTA concentrations at different percentages. In both yeast cell walls, the percentage of adsorption for all concentrations of OTA was higher than 200 µg/mL to about 100 µg/mL of yeast cell walls, except for the concentrations of 80 ng/mL. The highest adsorption capacity (89.3 ± 0.6) was observed when the highest concentration used YCW (200 µg/mL) and the concentrations of smaller OTA (10 ng/mL) in both adsorbents used. While the lowest adsorption capacity (8.9 ± 15.4) was analyzed occurred when the lowest concentration of YCW (100 µg/mL) and the highest concentration of OTA (1000 ng/mL).

Table 2. Percentage adsorption of ochratoxin A by two concentrations yeast cell walls.

Ochratoxin A concentrations (ng/mL)	Adsorption percentage (%)[a]			
	YCW1 (µg/mL)[b]		YCW2 (µg/mL)[c]	
	100	200	100	200
10	86.7 ± 2.9	88.8 ± 0.3	88.3 ± 11.5	89.3 ± 0.6
80	55.1 ± 0.9	51.7 ± 1.4	43.5 ± 13.3	43.3 ± 5.1
160	21.9 ± 9.6	24.4 ± 0.5	13.6 ± 0.7	23.9 ± 20.7
1000	17.0 ± 10.4	29.9 ± 2.8	8.9 ± 15.4	25.4 ± 43.7

[a] Mean ± SD.
[b] YCW1: yeast cell wall 1.
[c] YCW2: yeast cell wall 2.

DISCUSSION

The present study assessed the ability of two commercial YCW to adsorb OTA at pH 2 for 30 min and at 37 ± 0.5ºC.

In this study the YCW tested differ in their chemical compositions. Yeast wall cell 2 contains a higher percentage of mannans (21%) and β-glucans (23%) while the YCW1 containing 5.9% and 17.4% of mannans and β-glucans, respectively. The two YCW assayed containing different percentages of polysaccharides, were able to adsorb similar amounts of OTA. Furthermore, the relationship mannans/β-glucans generally does not influence the rate of adsorption of OTA. Similar results were reported by Yiannikours et al. (2006) who studied four yeast cell walls with different percentage of β-glucans and different relationship mannans/β-glucans and found no difference in the amount of OTA adsorbed. These results contrast with those obtained by Raju & Devegowda (2000), who reported that mannans can also bind mycotoxins such as OTA.

Huwig et al. (2001) showed that the adsorption of OTA was higher when replaced live yeast by YCW extracts. Other researchers showed that a mixture of 40% sterilized yeast and 60% fermentation residue of beer fermentation were efficient binders of OTA (8.6 mg/g) in an in vitro adsorption study (Bauer 1994). A recent report suggested that oenological strains of *Saccharomyces* sp. yeasts can be used for the decontamination of OTA in synthetic and natural grape juice (Bejaoui et al. 2004). Heat treated cells showed a high adsorption percentage (90% w/w) when compared to viable cells (35% w/w) indicating a physical nature of binding and

cell density played an important role in adsorption efficiency. But none of them takes into account the chemical composition of the YCW.

Based on chemical composition and physical nature of *S. cerevisiae* cell wall, it is reasonable to think that cell surface presents innumerable sites on its surface for physical adsorption of molecules (Shetty & Jespersen 2006). Cell surface sorption is a physico-chemical interaction between the toxin and functional groups of the cell surface, based on physical adsorption, ion exchange and complexation. Cell walls of microbial biomass are mainly composed of polysaccharides, proteins and lipids and offer abundant functional groups, such as carboxyl, hydroxyl, phosphate and amino groups, as well as hydrophobic adsorption sites such as aliphatic carbon chains and aromatic rings (Ringot et al. 2005).

Results from this work related to adsorption capacity of OTA with YCW allow predicting that other factor than 3D-structure and β-glucans and mannans could be involved.

Due to OTA toxicity and in order to assure human and animal health, this toxin should not be present in feed at least above the maximum permitted level. Both YCW exhibited high OTA binding capacity in vitro. Future studies could be conducted to test the in vivo binding ability to alleviate the toxic effects of OTA under field conditions. Both YCW are a promising mycotoxin adsorbent to be used in animal feed to prevent mycotoxicoses.

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