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A B S T R A C T

The objectives of this study were: to evaluate the use of dry distillery grain soluble extract - DDGse to produce yeast biomass and to obtain cell wall (CW), to use the CW as an aflatoxin B_1 (AFB$_1$) adsorbent, to study the variation in the composition and thickness of the CW under the influence of DDGse to evaluate their implication on the adsorption process using transmission electron microscopy (TEM) and fourier-transform infrared spectroscopy (FITR). The production of biomass and CW were variable. The CW thickness values showed that S. boulardii strain grown in yeast extract peptone dextrose (YPD) or DDGse medium, with no significant differences observed. The thickness of the CW for S. cerevisiae (RC012 and VM014) were increased when the cells were grown in DDGse medium, the thickness was almost double compared to the values obtained in YPD medium. The spectra IR of each CW in the two culture media shown regions corresponding to polysaccharides, proteins and lipids. Cells grown in DDGse medium adsorbed more AFB$_1$ than those grown in YPD. The CW adsorbed more AFB$_1$ than the same amount of whole cell. Future studies should be done to determine the type of carbohydrates and the relationship between chitin - beta glucans responsible for mycotoxin adsorption

1. Introduction

Yeasts are fairly simple in their nutritional requirements as they are able to metabolize a range of carbohydrates as carbon source. They require a reduced carbon source, various minerals, a supply of nitrogen, vitamins including biotin, pantothenic acid, thiamine and relatively small amount of oxygen (Halbandge, Vidyasagar, & Karuppayil, 2017). Additionally, yeast is advantageous because of its simple growth re- quirements, rapid cell division, and ease of genetic manipulation and availability of experimental tools for genome-wide analysis (Simon & Bedalov, 2004).

Yeasts are the main producer of biotechnological products world- wide, which exceeds the production, capacity and economic income of any other group of industrial microorganisms. Traditional industrial attributes of yeasts include their primary roles in many food fermen- tations, they have been used for biomass production, cultivated as rich sources of protein, minerals, vitamins (particularly B vitamins), and other nutrients for humans and animals (Anupama & Ravindra, 2000; Bekatorou, Psarianos, & Koutinas, 2006).

Certain yeast species have been used as prebiotic and probiotic agents for preventing or treating various intestinal, nutritional, and toxicological disorders. The main probiotic yeasts are Saccharomyces boulardii and S. cerevisiae (McFarland, 2017; Vieira, Teixeira, & Martins, 2013). Some of the properties that make both yeasts probiotic are the ability to traverse the gastrointestinal tract, help maintain and restore intestinal flora, they are non–pathogenic and have optimal growth at 37 °C. Also, they have the ability to restrict a variety of microbial pathogens. This probiotic yeast has received Qualified Presumption of Safety (QPS) status from The European Food Safety Authority (EFSA). Saccharomyces boulardii, is the only probiotic with clinical importance (Satyanarayana and Kunze, 2017).

The most important nutrients for yeasts are carbohydrates that serve for both, carbon and energy sources. The ability of yeasts to metabolize polysaccharides and complex carbohydrates is restricted to relatively few species. Utilization of starch is of particular interest for industrial production of yeast biomass (single-cell protein, SCP) from starchy agricultural wastes (Dhanasekaran, Lawanya, et al., 2011a, Dhanasekaran, Shanmugapriya, et al., 2011b). Different substrates have been used for the production of biomass as molasses, starch, cassava, Jerusalem artichoke, whey products, sulfite waste liquor, potato wastes, brewery wastes, and other waste streams from agricultural processes food processing, and industrial processes (Ozyurt & Deveci, 2004).

Saccharomyces sp. has received attention thanks to the biotechno- logical applications for enzyme production, for fermented foods and beverages, for biofuels, and for cell factory applications.

Saccharomyces sp. can be easily and rapidly grown in a diverse array of culture media at a low production cost. Many changes in carbohy- drate composition of cell wall occur when S. cerevisiae cells grow under various conditions. In particular, some agroindustrial wastes represent a good choice for microbial biomass biosynthesis because they are rich in carbohydrates and other nutrients (Sharma et al., 2014).

In recent years, the use of yeast cell wall has gained importance as adsorbents of mycotoxins, including aflatoxin B1 (Yianninkouris et al., 2003; Yiannikouris, François, Poughon, Dussap, Bertin, Jeminet, andJouany, 2004, Yianninkouris, Francois, Poughon, Dussap, Jeminet, and Jouany, 2004; Yiannikouris et al., 2006). The AFB1 is the most carci- nogenic and hepatotoxic mycotoxin that can be found in most food and cereals intended for human and animal consumption (Cao & Fan, 2011; Dhanasekaran, Lawanya, et al., 2011a, Dhanasekaran, Shanmugapriya, et al., 2011b). Yiannikouris et al., 2006 showed that adsorption of mycotoxins is correlated to the amount of D-glucans and chitin con- tained in yeast cell wall. In this way, the growth in certain carbon sources would allow to increase the thickness of the wall, with it the increase of carbohydrates, in order to improve the percentages of my- cotoxin adsorption. The present work combines the use of an agroin- dustrial waste (to give added value to this substrate), as a carbon source to increase the yeast cell wall of three strains of probiotic yeasts and then, to demonstrate the cell wall aflatoxin adsorption.

The objectives of this study were: 1) to evaluate the use of an agroindustrial waste such as Dry Distillery Grain Soluble Extract (DDGse) to produce yeast biomass and to obtain cell wall, 2) to use the cell wall as an AFB1 adsorbent, 3) to study the variation in the com- position of the cell wall under the influence of DDGse and thickness to evaluate their implication on the adsorption process.

2. Materials and methods

2.1. Yeast strain and growth conditions

The strains used in this study belong to the genus Saccharomyces. Three strains S. boulardii RC009, S. cerevisiae RC012 and S. cerevisiae VM014 with demonstrated probiotic properties were used. Strains were molecularly identified and deposited in National University of Rio Cuarto, Cordoba, Argentina (RC) collection centre (Armando et al.,
2011; Díaz-Vergara et al., 2017). The obtained sequences have been deposited in GenBank under accession numbers MH266045, MH266046 and MH266047 (see http://www.ncbi.nlm.nih.gov/ nucleotide).

The colony grown on malt extract agar (MEA) was inoculated into a tube with yeast extract-peptone-dextrose broth (YPD) and incubated at 30 °C for 12 h to be a pre-inoculum. From this pre-inoculum, a cell suspension (1 × 107 cells mL−1) was performed and each strain was inoculated into 100 mL of YPD broth (as a control culture medium) and
100 mL of broth based on DDGse and incubated at 30 °C for 24 h with shaking (150 rpm).

2.2. Preparation of the dry distillery grain soluble extract

The DDGs were obtained from a local bio-ethanol producing plant. The DDGse was prepared by adding water (300 mL + 75 g DDGs) to obtain a 25% DDGse that was left at fluent steam for 20 min and then filtered. To 100 mL of the filtered extract, 1 g of yeast extract and 0.1 g of potassium phosphate was added. The DDGse was then autoclaved at
121 °C, 1 atm for 15 min.

The estimation of reducing sugars from the DDGse medium was determined using the dinitrosalicylic acid (DNS) method (Miller, 1959). Seventy five (75 g) of NaK tartrate and 4 g of NaOH were weighed. The NaOH was dissolved in 200 mL of distilled water and the NaK tartrate was added slowly under stirring. 3.5 DNS acid (0.25 g) was added slowly to a volumetric flask and filled with distilled water to 250 mL.

One-hundred µL of the sample with 1 mL of the DNS reagent were mixed and incubated in a water bath at 100 °C for 10 min. The tubes were cooled and measured in a spectrophotometer at 570 nm. The ca- libration curve was made using different glucose concentrations (0.004,0.4, 0.8 and 4 g.L−1). All samples were analyzed in duplicate.

Table 1
Biomass and cell wall production (g L−1) of Saccharomyces boulardii RC009, S. cerevisiae RC012 and S. cerevisae VM014 obtained from two different culture media.

Yeast strains	Culture media	Biomass production (g L)	Cell wall production (g L)	(%)
S. boulardii (RC009)	YPD	4.02	1.19	29.6
	DDGse	3.15	0.22	6.98
S. cerevisiae	YPD	5.00	1.85	37
(RC012)	DDGse	4.02	0.65	16. 2
S. cerevisiae	YPD	4.89	0.108	2.22
(VM014)	DDGse	3.69	0.761	20.6

2.3. Biomass production and cell wall preparation

Biomass production was performed following the methodology of Nguyen, Fleet, and Rogers (1998), with some modifications.

Cells used for cell wall preparation were cultured to late exponential phase (about 18 h) in YPD and DDGse broth. The cultures were in- cubated at 28 °C in an orbital shaker at 150 rpm. Yeast cells were har- vested by centrifugation at 5000 rpm for 10 min, washed three times with water to remove traces of culture medium and then twice more with cold (4 °C) 0.1 M sodium phosphate buffer, pH 8.5. The biomass was dried at 60 °C in forced air oven until constant weight.

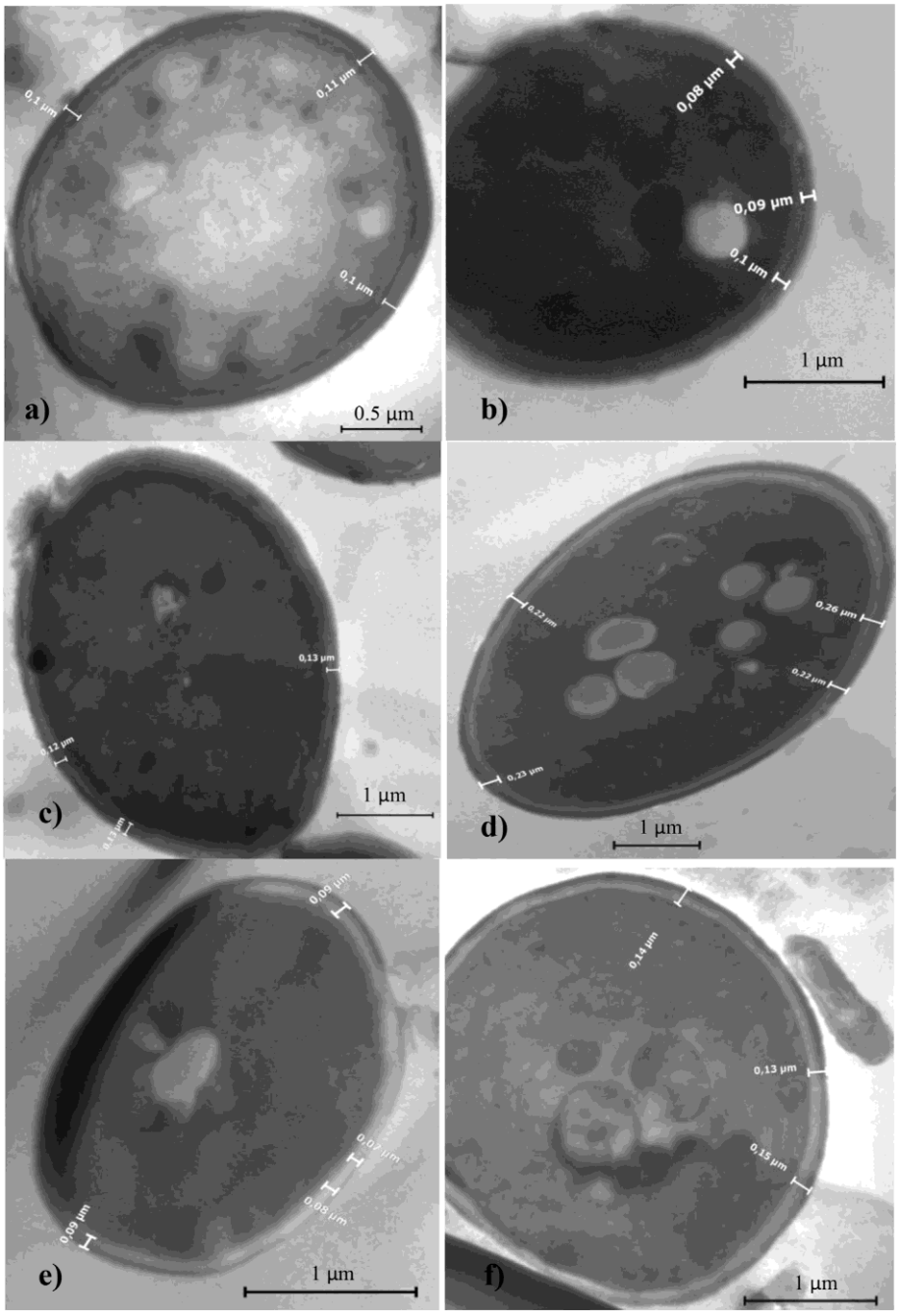


Fig. 1. The TEM photographs of the cell wall ultrastructural analysis of a) Saccharomyces boulardii (RC009) in YPD, b) S. boulardii in DDGse, c) S. cerevisae RC012 in YPD, d) S. cerevisiae RC012 in DDGse, e) S. cerevisiae VM014 in YPD and f) S. cerevisiae VM014 in DDGse.

Yeast cells were suspended in 0.1 M sodium phosphate buffer, pH 8.5, and an equal volume of glass beads (0.45 mm diameter). They were cooled to 4 °C and broken by mechanical shaking for 30 s, after which the homogenate was cooled and disrupted for another 30 s. This procedure was repeated five times. The glass beads were removed from the homogenate by decanting, and the cell walls were separated by centrifugation at 5000 rpm for 15 min. The cell walls were washed five times with 0.1 M phosphate buffer, pH 8.5, and then washed a further four times with distilled water. The temperature was kept below 4 °C during all operations. The cell wall was dried at 60 °C in forced air oven until constant weight.

2.4. Ultra-structural analysis of yeast cells

The influence of carbon sources on the ultrastructure of the cell wall was observed with electron microscopy. The yeast pellet was obtained by centrifugation, to determine the cell wall thickness and the cell wall thickness/cell wall ratio, the Transmission Electron Microscopy (TEM) technique was used.

Samples were homogenized for 30 min and centrifuged a 10,000 rpm for 10 min. The dried pellet was processed for TEM (Bozzola & Russel, 1999). All samples were pre-fixed in 3% (w.v−1) glutaraldehyde in 0.1 M sodium phosphate buffers, pH 7.2 for 3 h a room temperature followed by thorough washing with phosphate
buffer. Fixed materials were then post-fixed in 1% aqueous osmium tetroxid for 3 h a room temperature. Dehydration of samples was achieved by transferring to vials containing graded water– acetone series (10% steps for 30–90% each of 60 min, 100% for 180 min and finally 100% overnight). Dehydrated specimens were embedded with EMbed 812 and acetone 100% by 24 h, then were embedded with EMbed 812 with 1.5% hardening agent, DMP-30 at 60 °C by 24 h. The ultra-thin sections (± 60 nm) were cut and placed on cupper grids, counterstained with saturated uranyl acetate and aqueous lead citrate. The sections were examined in transmission electron microscope Elmiskop 101 (Siemens, Germany).

Table 2
Analysis of variance of the yeast strains (S), the culture media studied (CM) and their interaction for diameter and thickness cells.

Source of variation	SS	DF	SM	F	p-Value
Diameter of whole cell					
Strain	0.42	2	0.21	15.20	0.0001
Culture media	5.9 × 10−4	1	5.9 × 10−4	0.04	0.8377
S*CM	0.35	2	0.18	12.90	0.0003
Thickness of cell wall					
Strain	0.24	2	0.12	107.27	< 0.0001
Culture media	0.11	1	0.11	95.51	< 0.0001
S*CM	0.18	2	0.09	78.94	< 0.0001

SS: sum of square; DF: degrees of freedom; SM: square medium.

Table 3
Ultrastructural analysis of Saccharomyces boulardii RC009, S. cerevisiae RC012 and S. cerevisiae VM014: relationship between cell wall thickness/cell diameter
(μ m).

Yeast strain	Media	Ultrastructural analysis		
		Diameter of whole cells (μm)	Thickness of cell wall (μm)	Cell wall thickness/cell diameter/ (μm)
S. boulardii	YPD	3.84 ± 0.21	0.126 ± 0.018abc	0.0328
RC009	DDGse	3.05 ± 0.33	0.095 ± 0.013ab	0.0311
S. cerevisiae	YPD	3.94 ± 0.74	0.130 ± 0.036bc	0.0339
RC012	DDGse	3.63 ± 0.37	0.277 ± 0.064d	0.0759
S. cerevisiae	YPD	2.31 ± 0.06	0.088 ± 0.013a	0.0381
VM014	DDGse	3.27 ± 0.44	0.144 ± 0.023c	0.0440

2.5. Infrared spectroscopy

To verify the variation in the composition of the cell wall under the influence of the different carbon sources (YPD and DDGse) infrared spectroscopy (IR) was performed.

The dried cell wall was mixed with KCl (1 mg sample with 200 mg KCl). This mixture was ground in an agate mortar, finally making the tablet was performed under pressure (≈15 ton cm−2) and applying dynamic vacuum for 15 min.

Measurements were performed in a Nicolet FTIR Impact 400 spec- trometer. For data acquisit8731ion and processing the software OPUS was used. The spectra were the result of an accumulation 200 measure- ments, to increase signal/noise ratio, and were measured between 4000 and 400 cm−1 with a resolution of 4 cm−1.

2.6. Aflatoxin B1 production

Aflatoxins (AFs) for in vitro assays were produced via the fermen- tation of rice by A. parasiticus NRRL2999. The sterile substrate placed in Erlenmeyer flasks was inoculated with 2 mL of the mould's aqueous suspension containing 106 spores.mL−1. The spores were counted using a Neubauer camera. Cultures were allowed to grow for 7 days at 25 °C

in the dark. On the 7th day, Erlenmeyer flasks were autoclaved and the culture material was dried at 40 °C in a forced air oven for 48 h. AFs

were extracted with chloroform following the procedure described in AOAC (1994). The total AF content in the extract of the culture was determined by High Performance Liquid Chromatography (HPLC) ac- cording to Trucksess, Stack, Nesheim, Albert, and Romer (1994).

2.7. Adsorption test of live yeasts

After the incubation time previously described, 1 mL (107 cells mL−1) was added to a microtube. The suspension was cen- trifuged to obtain a pellet of cells, discarding the supernatant. Cells were washed with distilled water to remove traces of YPD or DDGse broth. A cell suspension was made which was first treated with pH 2 solution (without toxin) and then a concentration of toxin (150 ng.mL−1 AFB1) contained in solution pH 8 was added, simulating the gastrointestinal tract (GIT). Controls of AFB1 and each pH solution were performed. All treatments were performed in duplicates. The pH 2 (50 mL of potassium chloride 0.2 M and 13 mL of hydrochloric acid 0.2 M) and pH 8 (100 mL of 0.1 M KH2PO4 and 93.4 mL of 0.1 M NaOH) solutions contained 150 ng/mL of AFB1. 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.

The mixture of cells and AFB1 was homogenized and incubated for 40 min at 37 °C at 100 rpm, simulating the GIT conditions of pigs. After the incubation period, the mixture suspensions were centrifuged and the supernatant containing the free toxin was transferred to other microtube.

2.8. Detection and quantification of aflatoxin B1

Aflatoxin B1 detection and quantification from each sample were performed by HPLC according to the methodology proposed by Trucksess et al. (1994). An aliquot (200 µL) was derivatized with 700 µL trifluoroacetic acid/acetic acid/water (20:10:70 v.v−1). Chromato- graphic separations were performed on a reverse-phase column (Silica Gel, 150 9 4–6 mm id., 5-µ particle size; VARIAN, Inc., Palo Alto, CA, USA). Acetonitrile/methanol/water (1:1:4 v.v−1) was used as mobile phase at a flow rate of 1.5 mL/min. Fluorescence of AF derivatives was recorded at excitation and emission wavelengths of ʎ 360 and ʎ 460 nm, respectively. Quantification of AFB1 was performed by mea- suring the area and its extrapolation to a calibration curve obtained using solutions of AFB1 standards.

The method was validated for linearity, accuracy, LOD and LOQ. Linearity of the method was tested by injecting three replicates (20 µL) of three levels of AFB1 standard solutions (5–50 ng/mL). The accuracy of the method was determined by a recovery assay as described above and the average content of AFB1 obtained was used to calculate the recovery percentage. The limit of detection (LOD) and limit of quanti-fication (LOQ) for AFB1 were calculated based on signal-to-noise (S/N) ratios of 3:1 and 15:1, respectively, by were experimentally obtained injecting standard dilutions with the corresponding S/N ratio.

2.9. Yeast cell wall adsorption test

The weight of the wall used for the tests was determined by the percentage of food additives (0.1%) added to feed usually intended for animal production.

Assays were performed in the same way as in the adsorption assay using whole cell. A wall suspension was also made which was first treated with pH 2 solution (without toxin) and then, a concentration of toxin (150 ng.mL−1 AFB1) contained in pH 8 was added, simulating the GIT.

Aflatoxin B1 detection and quantification from each sample were performed by HPLC according to the methodology proposed by Trucksess et al. (1994), that was previously described.

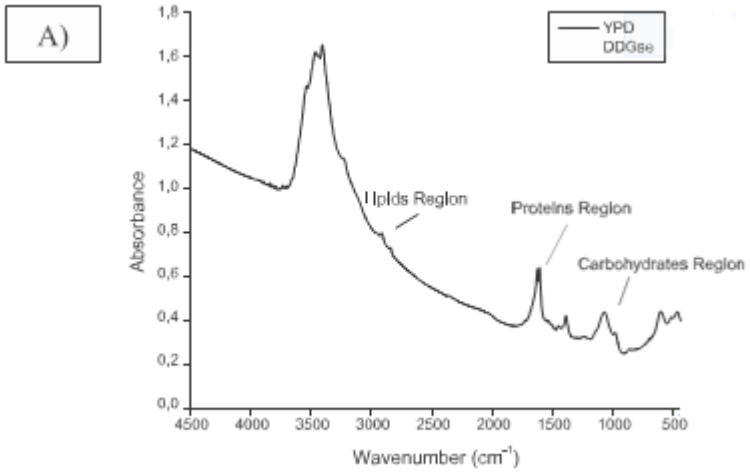
3. Statistical analysis

The assays were performed in triplicate for each treatment. The mean values of biomass production and cell wall (g.L−1), and adsorp- tion assays were analyzed using the analysis of variance (ANOVA). Means were compared using Fisher's protected LSD test (Quinn & Keough, 2002).

4. Results

4.1. Biomass and yeast cell wall production

Biomass production (g.L−1) and yeast cell wall production (%) are shown in Table 1. The production of biomass was variable. Sacchar- omyces boulardii RC009 and S. cerevisiae RC012 strains, both isolated from the porcine environment, showed higher production of biomass and cell wall using YPD medium. While, S. cerevisiae VM014 strain isolated from whey obtained higher biomass production using YPD medium (4.89 g.L−1); however, the cell wall percentage was higher that using DDGse medium (20.6% wall).



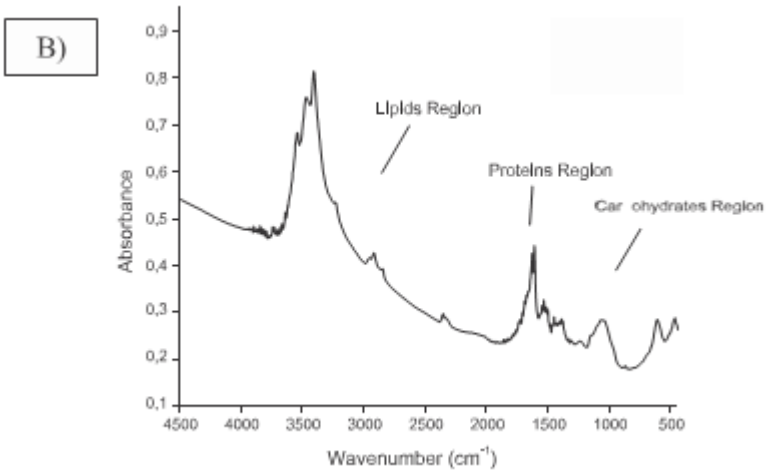


Fig. 2. FITR spectra of cell wall of grown in different culture media. A) Saccharomyces boulardii (RC009), B) S. cerevisiae (RC012), C) S. cerevisiae (VM014).

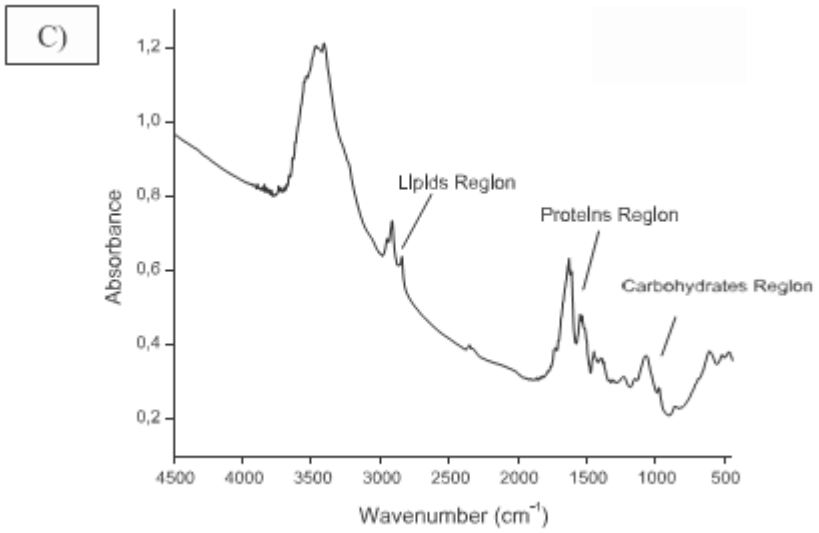


Fig. 2. FITR spectra of cell wall of grown in different culture media. A) Saccharomyces boulardii (RC009), B) S. cerevisiae (RC012), C) S. cerevisiae (VM014).

Table 4
Analysis of variance of the yeast strains, the adsorption of the whole cells and the cell wall and the culture media studied.

Table 4

Source of variation	SS	DF	SM	F	p-Value
Strain	34,115,252.95	2	17,057,626.47	1.33	0.2789
Culture media	63,457,554.30	1	63,457,554.30	4.95	0.0335
Whole cell - cell wall	11,299,423,897.23	1	11,299,423,897.23	881.69	< 0.0001

SS: sum of square; DF: degrees of freedom; SM: square medium.

4.2. Ultra-structural analysis of the cell wall by transmission electron microscopy

The morphology of the yeast cell wall determined by TEM is shown in Fig. 1. The wall of the strains is often described in terms of three layers, namely, an outer electron-dense layer, an adjacent less-dense layer and another dense layer that borders the plasma membrane, as can be observed in the TEM micrographs in Fig. 1.

The diameter of the cell and thickness of cell wall of the three strains were analyzed statistically with ANOVA ($p < .0001$) (Table 2). This study showed that there were significant differences between strains when the cell wall diameter was analized; there were no significant differences between the used culture media; however, the in- teraction between strains and culture media was highly significant. When the cell wall thickness was analized, significant differences between the strains, the culture media and the interaction between them were observed.

Table 3 shows the cell diameter and cell wall thickness mean values for S. boulardii RC009, S. cerevisiae RC012 and S. cerevisiae VM014 for each culture medium studied. When the yeast strains growing at different culture media were analyzed for the cells diameter, no significant differences were observed, the average cell diameter values ranged from 2.31 µm for S. cerevisiae VM014 to 3.94 µm for S. cerevisiae RC012, both grown in YPD. The cell wall thickness values showed that S. boulardii strain grown in YPD or DDGse medium, with no significant differences observed. The thickness of the cell wall for S. cerevisiae RC012 and S. cerevisiae VM014 were increased when the cells were grown in DDGse medium, the thickness was almost double compared to the values obtained in YPD medium.

4.3. IR spectroscopy analyzed

The spectra of each cell wall in the two culture media are shown in Fig. 2, where three (3) regions corresponding to polysaccharides (950–1185 cm−1), proteins (1480–1700 cm−1) and lipids (2840–3000 cm−1), were observed.

The presence of carbohydrates, where β-glucans are present, was high for the strain S. boulardii RC009 when the cells were grown in YPD broth, while the cells walls of S. cerevisiae RC012 and S. cerevisiae VM014 grown in DDGse broth showed higher carbohydrate amounts compared to those obtained in YPD medium.

4.4. Adsorption of AFB using whole cell and cell wall under simulated gastrointestinal pH solution1

For validation of method, the minimal amounts of AFB1 detectable and quantifiable with this method were 0.008 ng and 0.04 ng, respec tively. The LOD and LOQ for this method were 0.4 and 2 ng AFB1/mL of sample, respectively. Linear correlation (r2 > 0.968) was obtained between peak area and concentration of AFB1 standard solutions ran- ging from 0.02 to 0.22 ng. The average AFB1 recovery from pH 2 and pH 8 was 101,33% and 97,32, respectively.

The adsorption values of AFB1 obtained from the three (3) strains were analyzed statistically with ANOVA ($p < .05$). The statistical study showed that there were no significant differences between the strains.
The three strains showed similar behavior. However, there were significant differences between the culture media (YPD and DDGse) and between the whole cell and the cell wall (Table 4). Cells grown in DDGse medium adsorbed more AFB1 than those grown in YPD. The cell wall adsorbed more AFB1 than the same amount of whole cell. Adsorption of AFB1 using whole cells and cell wall of S. boulardii RC009, S. cerevisae RC012 and S. cerevisae VM014 under simulated GIT pH solution are shown in Table 5.

The adsorption of AFB1 using the whole cell of S. boulardii RC009 was higher when they were grown in DDGse medium. While, there was no significant difference for the adsorption of AFB1 using S. cerevisiae (RC012 and VM014) in both media. The mean adsorption values of the whole cells grown in YPD were similar among the three (3) studied strains and lower than those obtained from DDGse medium.

In relation to the cell wall use of each yeast species, they adsorbed almost 10 times more than using the same amount of whole cell. However, there are no significant adsorption differences between the cell walls of each species and for each culture medium.

Table 5
Adsorption of AFB_1 using whole cells and cell wall of Saccharomyces boulardii (RC009), S. cerevisiae (RC012 and VM014) in simulated gastrointestinal pH solution.

Yeast strain	Culture media	Whole cell		Cell wall	
		Adsorption media ± SD (µg/g)	LSD	Adsorption media ± SD (µg/g)	LSD
S. boulardii (RC009)	YPD	3.77 ± 1.25^{ab}	a	40.47 ± 5.69	b
	DDGse	5.72 ± 0.79^{c}		43.82 ± 3.53	
S. cerevisiae (RC012)	YPD	4.13 ± 1.29^{ab}		37.49 ± 1.54	
	DDGse	5.01 ± 0.22^{bc}		37.85 ± 1.76	
S. cerevisiae (VM014)	YPD	3.43 ± 0.54^{a}		35.52 ± 9.28	
	DDGse	4.42 ± 0.40^{abc}		43.93 ± 3.11	

YPD: yeast extract – peptone – dextrose broth. DDGse: dried distiller grains with solubles extract. The same letters do not indicate significant differences. Analyses were performed for each column separately according to Fisher's minimal significant difference test (LSD) with a $P < .05$.

5. Discussion

The present study evaluates the use of an agro-industrial waste (DDGse) as a carbon source for the production of biomass and cell wall from S. boulardii RC009, S. cerevisiae RC012, S. cerevisiae VM014 to be used as AFB1 adsorbent. In addition, the composition and the cell wall thickness were analyzed to evaluate their implication in the adsorption processes.

In particular, agro-industrial wastes represent a good choice for microbial biomass biosynthesis because they are rich in carbohydrates and other nutrients (Sharma et al., 2014).

The chemical composition of many fungal cell walls is known. The biochemistry and molecular genetics of biosynthesis have been reviewed (Aguilar Uscanga & François, 2003; Lipke & Ovalle, 1998). Cid et al. (1995) have studied the biosynthesis of cell wall components, with different carbon sources and other components to maximize the enzymatic activity responsible for the synthesis of beta glucans mainly and other cell wall components.

In this study, a basic medium such as YPD and an alternative low cost medium such as DDGse were used as carbon source since the composition of the cell wall may vary under different growth conditions, including the type of culture, carbon source, temperature, pH and aeration (Aguilar Uscanga & François, 2003; Naruemon et al., 2013). The results from the three yeast strains studied showed that YPD medium was better than DDGse in the production of biomass. It is important to note that the amount of glucose from the DDGse medium was 2.67 g.L−1 while the amount of glucose in YPD was 20 g.L−1. The DDGse medium proved to be recommendable for biomass and cell wall production. The produced biomass was used for the cell wall extraction in order to demonstrate the AFB1 adsorption.

In general, the cell wall of Saccharomyces cerevisiae, is about 70 nm thickness that represent 20% of the whole cell's weight (Walker, 1999). The percentage of cell wall of S. boulardii RC009 in YPD (29.6%), S. cerevisiae RC012 in YPD (37%) and S. cerevisiae VM014 in DDGse (20.6%) were similar to those extracted by Nguyen et al. (1998) and Francois (2006), who obtained values of 29% and 22.7% cell wall, re- spectively. However, Nguyen et al. (1998) used a medium based on yeast extract (5 g.L−1) and glucose (50 g.L−1) and obtained values of 29.5 and 32.5 (%). Aguilar Uscanga, Solis Pacheco, and François (2005) studied the variation of the composition of polysaccharides contained in the cell wall of S. cerevisiae using different sources of carbon (glucose, mannose, galactose, sucrose, maltose and ethanol). The percentage of cell wall as dry weight was 10% for the culture made with 25% ethanol in the sucrose culture. The values obtained in this study for S. boulardii RC009 in DDGse and for S. cerevisiae VM014 were below 10% in comparison with the values obtained by Aguilar Uscanga et al. (2005) and Nguyen et al. (1998). S. cerevisiae RC012 values in DDGse (16.2%) were similar to those obtained by Francois (2006) using synthetic medium only with glucose (16.5%) or mineral medium with glucose (16.3%).

In this work, the composition of the cell wall using FTIR was studied and the thickness of the wall was determined by TEM. Fourier trans- form infrared spectroscopy can be applied as a useful tool for the analysis of entire yeast cells providing a fast, effective, reagent-free, and simple method for the determination and quantification of carbohy- drate composition of yeast (Kuligowski, Quintás, Herwig, & Lendl, 2012; Plata, Koch, Wechselberger, Herwig, & Lendl, 2013). The IR technique allows to study the total carbohydrate variation of the cell wall under the influence of two carbon sources. The yeast cell wall spectrum shows three characteristic regions such as carbohydrates, proteins and lipids, which agree with previous works (Adt, Toubas, Pinon, Manfait, & Sockalingum, 2006; Ahmad et al., 2010; Naruemon et al., 2013; Plata et al., 2013). The use of YPD increased the absor- bance of carbohydrates only in S. cerevisiae RC012. While the use of DDGse increased the polysaccharide region in S. boularii RC009 and S.

cerevisiae VM014. The adsorption of mycotoxins is correlated to the amount of D-glucans and chitin contained in yeast cell wall (Yiannikouris et al., 2006). Future studies would need to be focused on determining the different types of carbohydrates.

In this study, the relationship between the thickness of the cell wall and the cell diameter was evaluated using electron microscopy to de- termine the proportion of cell wall present in the strain. The relation- ship between cell diameter and wall thickness shows an accurate esti- mate of the cell wall content. The diameter of the cells was similar between the two culture media studied and the three strains. The use of DDGse increased the thickness of the cell wall for strains of S. cerevisiae RC012 and VM014. On the other hand, there were no differences between the culture media tested in the S. boulardii strain RC009 in relation to the thickness of the wall. In this study, the use of DDGse as a carbon source could replace synthetic media such as YPD for the pro- duction of biomass giving an added value also for the production of cell wall, where in some strains of yeasts it was demonstrated that it increases the thickness of the wall, which was used for the adsorption of toxin.

The yeast cell wall in particular offers a plethora of possibilities. One of the alternatives is the use as mycotoxin adsorbents. Several studies have been reported on the biodegradation and adsorption of mycotoxins using different yeast species, mainly Saccharomyces cerevisiae and others such as Rodhotorula sp., Pichia kudriavzevii, Clavispora lusitaniae, Candida krusei and Pichia anomalous, Candida guilliermondii, Candida intermedia, Candida lusitaniae (Armando et al., 2012; Fiori et al., 2014; Magnoli et al., 2016; Var, Erginkaya, & Kabak, 2009; Yianninkouris et al., 2003; Yin, Yan, Jiang, & Ma, 2008). There are no previous studies related to the adsorption of AFB1 by S. boulardii RC009, using the cell wall.

The adsorption values of AFB1 found in our study are similar to Dakovíc et al. (2008) using copper modified montmorillonite (0.066 g.g−1) as an adsorbent and lower than those obtained by Galvano et al. (1997) using activated carbon (0.12 g.g−1). Pereyra, Cavaglieri, Chiacchiera, and Dalcero (2012) studied the adsorption of AFB1 with walls of commercial yeasts applying mathematical models to explain the type of interaction of the toxin with the adsorbent. They found values of 0.29 ± 0.01 (g.g−1) at 0.40 ± 0.1 (g.g−1) for pH 2 and 0.061 ± 0.003 (g.g−1) at 0.15 ± 0.01 (g.g−1) at pH 6.

Yeast cell wall is mainly composed of polysaccharides, proteins and lipids that offer different functional groups (carboxyl, hydroxyl, phosphate an amine groups) as well as hydrophobic adsorption sites, such as aliphatic chains and aromatic carbon rings for the interaction with the toxin (Jouany, Yiannikouris, & Bertin, 2005; Ringot et al., 2005). Future studies should be done to determine the type of carbohydrates there are and the relationship between chitin - beta glucans responsible for mycotoxin adsorption.

The use of yeast cell walls as mycotoxin adsorbents is a promising and economical strategy to reduce the exposure of animals (and therefore humans) to mycotoxins. These would be incorporated as ad- ditives in animal feed to reduce the absorption of gastrointestinal mycotoxins.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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