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Culture medium and gastrointestinal environment positively influence the *Saccharomyces cerevisiae* RC016 cell wall polysaccharide profile and aflatoxin B_1 bioadsorption

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ABSTRACT

The objective was to study the influence of culture medium and gastrointestinal environment on *Saccharomyces cerevisiae* RC016 cell wall polysaccharides profile and the aflatoxin B_1 (AFB_1) adsorption. Probiotic *Saccharomyces cerevisiae* RC016, isolated from the pig small intestine, has previously shown efficient mycotoxins adsorption *in vitro* including AFB_1. In addition, it was able to survive under the gastrointestinal (GI) tract conditions and did not cause *in vitro* and *in vivo* genotoxicity or cytotoxicity. In this work, transmission electron microscopy (TEM) was used to show ultrastructural variations in cell morphology and, infrared spectroscopy (IR-frequency range 4000–500 cm^{-1}) was used to show variations in the spectra of yeast cells cultured in different culture media, Yeast extract Peptone Dextrose (YPD) medium and Dried distillers grains (DDG) medium (after passing the simulated GIT). The cell wall thickness in the DDG medium was the highest (68%-$p \leq 0.0001$). Infrared spectra had a similar spectral pattern for all treatments, however, bands from DDG medium had greater absorption intensity than those obtained from YPD and in turn, increased after the GI tract passage. *S. cerevisiae* RC016 showed important AFB_1 adsorption in the simulated intestinal fluid (97.7% in DDG). The optimization of nutritional conditions coupled with the use of spectrophotometric tools allowed increasing, in a controlled manner, the main components of the cell wall responsible for adsorbing mycotoxins simulating the GI tract. Consequently, it could be possible to control the functional properties (probiotic and mycotoxin adsorbents) after scaling the yeast biomass production with low-cost substrates, allowing its effective application as a food additive.

1. Introduction

The yeast cell wall has a two-layer structure composed mainly by polysaccharides (β-glucans, mannans, and chitin) that contribute between 20% and 30% of the cell weight (Aguilar-Uscanga & Francois, 2003; Magnelli, Cipollo, & Abeijon, 2002). The yeast cell wall is not a static entity but rather dynamically remodeled in response to different environmental conditions (Klis, Boorsma, & De Groot, 2006; Liu, Wang, Liu, & Tan, 2008). They respond to environmental stress by changes in the molecular architecture, modifying the relationship between cell wall polysaccharides and increasing the amounts of several cell wall proteins. As a consequence, the cell wall is remodeled in an attempt by the cell to survive (Arroyo, Bermejo, García & Rodríguez-Peña, 2009).

During industrial production, the amount and structural properties of cell wall components such as β-glucans, mannan, and chitin may vary depending on culture conditions, including pH, temperature, oxygenation, concentration or the nature of the carbon source. This may modify some functional properties, such as antitumor, immunostimulatory and antioxidant activities (Schar-Zammaretti, Dillmann, D'Amico, Affolter, & Ubbink, 2005; Deepika, Green, Frazier, & Charalampopoulos, 2009). Oligosaccharides such as β 1–3 glucans and mannan proteins constitute the binding site to mycotoxins and metal ions (Shetty, Hald, & Jespersen, 2007). Aflatoxins (AF) are toxic, low molecular weight secondary metabolites produced by fungal

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species belonging to *Aspergillus* genera (*A. flavus* and *A. parasiticus*). They are classified by the International Agency for Research on Cancer (IARC) as group 1 carcinogens (that is, carcinogenic to humans) (IARC, 2002). The main AF, AF B_1 (AFB_1), AF B_2 (AFB_2), AF G_1 (AFG_1) and AF G_2 (AFG_2) are ubiquitously found in many agricultural products. The food and feed industries are subject to significant economic losses associated with the mycotoxins prevention, control and detoxification procedures (Synytsya & Novak, 2014; Wu, Groopman, & Pestka, 2014).

The yeast cell wall components (β-1-3 glucan and mannan-proteins) exhibit a variety of sites for mycotoxins including AF, fumonisins, zearalenone (ZEA) and ochratoxin A with different binding mechanisms (hydrogen bonds, ionic or hydrophobic interactions) responsible for the mycotoxins adsorption in agricultural products (McCormick, 2013; Yiannikouris, André, Poughon, & François, 2006; Yiannikouris, François, Poughon, & Dussap, 2004).

The application of yeasts has great potential to reduce the economic damage caused by toxigenic fungi in agriculture by decreasing the availability of mycotoxins at the level of the GI tract (Watson & Preedy, 2015). The use of microorganisms capable of adsorbing mycotoxins includes bacteria (*Lactobacillus* and *Bifidobacterium*) and yeasts (*S. cerevisiae*) that are widely used in food fermentation (Shetty et al., 2007).

Yeast biomass is a good source of adsorbent material for mycotoxins. However, there are few studies on the changes that occur at the macromolecular and the cell wall ultrastructure level when cells are produced at large scale using low cost substrates. During cell growth, the culture medium composition can influence the cell wall morphology and thickness modifying functional properties such as binding to mycotoxins. Probiotic *Saccharomyces cerevisiae* RC016, isolated from the pig small intestine, has previously shown efficient mycotoxins adsorption *in vitro* including AFB_1 (Armando et al., 2012, 2011; Armando, Galvagno, Dogi, & Cerrutti, 2013). In addition, it was able to survive under the GI tract conditions and did not cause *in vitro* and *in vivo* genotoxicity or cytotoxicity (Armando et al., 2011; Dogi et al., 2011; González Pereyra et al., 2014). Now, the objective of this work was to study the cell wall ultrastructural variability through the carbohydrate profile when *S. cerevisiae* RC016 was grown in different culture media and under simulated GI tract conditions, and consequently, the influence on the AFB_1 adsorption was evaluated.

2. Materials and methods

2.1. Yeast strain and cultural conditions

Saccharomyces cerevisiae strain RC016 was isolated from pig intestine, and deposited in National University of Rio Cuarto, Cordoba, Argentina (RC) collection centre. Stock cultures were maintained at -80 °C in 30% (v/v) glycerol. Working cultures were prepared from frozen stocks by two transfers on Yeast extract – Peptone - Dextrose (YPD) broth (5 g yeast extract, 5 g peptone, 40 g dextrose, 1000 mL water) and incubation at 28 °C for 24 h on an orbital shaker (180 rpm). Morphological and molecular characteristics of this strain were informed previously (Armando et al., 2011).

The yeast growth was evaluated in a 5 L stirred tank bioreactor (BIOFLO 2000; New Brunswick Scientific, USA), operated in batch mode at 28 ± 2 °C at 250 rpm 2 vvm aeration (6 L/min), with 3 L of Dried Distillers Grains extract (DDG) medium prepared by adding water (300 mL + 75 g DDG) to obtain a 25% DDG that was left at fluent steam for 20 min and then filtered. The extract was autoclaved at 121 °C, 1 atm for 15 min (Fochesato et al., 2018). This substrate was obtained from a local bioethanol production plant. Its composition is reported in Table 1. The extract was previously sterilized in an autoclave at 121 °C, 1 atm for 15 min. The reactor was inoculated (10% of the work volume) with *S. cerevisiae* (2×10^7 CFU/mL). The dissolved oxygen concentration at the beginning of the experiment was 100% saturation. Foam production was controlled by the addition of Antifoam 289 antifoam (Sigma-Aldrich, St. Louis, MO, USA). Samples were taken at 24 h for the adsorption tests.

Table 1
Dried distilled grains (DDG) centesimal composition.

Dried distilled grains (DDG)	
Components	Percentage
Dry matter	35.97
Water	64.03
Total protein	10.52
Total fats	4
Total fibre	3.08
Neutral detergent fibre (NDF)	3.76
Acid detergent fibre (ADF)	8.98
Starch	1.79
Sulphur	0.23
Phosphorus	0.32
Ashes	2.28

The YPD medium was also evaluated in a bioreactor that worked at the same conditions detailed above.

2.2. Simulated gastrointestinal tract solutions composition

Simulated GI tract conditions were prepared as follows: artificial salivary solution: lysozyme 2 mg/mL (Sigma 47700 U/mg) in saline solution pH 6.5; simulated gastric juice: NaCl 125 mmol, KCl 7 mmol, $NaHCO_3$ 45 mmol, pepsin 3 g/L (Sigma 427 U/mg) adjusted to pH 3 with HCl; and artificial intestinal fluid (IF): trypsin 1 mg/mL (Fluka 11531 U/mg), chymotrypsin 1 mg/mL (Fluka 80 U/mg), bile salts 0.3% (w/v) adjusted to pH 8 with NaOH 5 mmol/L (Fochesato et al., 2019).

2.3. Ultrastructural analysis of yeast cells by transmission electron microscopy

Yeast pellets grown in two culture media, YPD and DDG, and after the passage through the GI tract solutions were obtained by centrifugation following the methodology proposed by Armando et al. (2012). Samples of *S. cerevisiae* RC016 were then fixed in 2.5% glutaraldehyde in 0.2 M S-collidine pH 7.4; a post fixation was performed in 1% osmium tetroxide in 0.2 M S-collidine pH 7.4 and then, dehydrated under increasing concentrations of acetone, embedded in epoxy resin Embed 812 and sectioned in an ultramicrotome (Sorvall MT-1A, DuPont, USA). These ultrathin sections (60 ηm) were cut and placed in copper gratings, contrasted with saturated uranyl acetate and aqueous lead citrate. The sections were examined *by transmission electron microscopy* (TEM) with a transmission electron microscope JEM 1200 ExII (JEOL, Japan), using a Digital Micrograph TM (Gatan, Inc., Japan) software.

2.4. Infrared spectroscopy

Infrared spectroscopy (IR) was performed to determine the carbohydrates present in *S. cerevisiae* RC016 under the influence of different carbon sources (YPD and DDG) during growth and then passed through the GI tract according to the methodology proposed by Pereyra et al. (2018). The whole cells were mixed with KBr (1 mg sample/200 mg KBr). This mixture was crushed in an agate mortar, finally causing the tablet to be made under pressure (≈15 ton/cm^{-2}) and applying a dynamic vacuum for 15 min.

The measurements were made on a Nicolet FTIR Impact 400 spectrometer. The OPUS software was used to acquire and process the data. The spectra were the result of an accumulation of 200 measurements, to increase the signal-to-noise ratio, and were measured between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.5. Effect of simulated gastrointestinal conditions on aflatoxin B_1 binding

The samples collected after fermentation in bioreactor were used to carry out the AFB_1 binding assay, from the cell pellet obtained by centrifugation (5000 g, 10 min) and washed with phosphate buffered saline (PBS, pH 7). The concentration of yeast pellet (1×10^7 cells/mL) was determined using a haemocytometer.

2.6. Aflatoxin B_1 solutions

Aflatoxin was produced according to the methodology proposed by Magnoli et al. (2018). This solution was used to contaminate each of the simulated solutions of the GI tract such as salivary solution (SS), gastric juice solution (GJS) and intestinal fluid solution (IFS). The concentration of AFB_1 in each solution was confirmed by High-Performance Liquid Chromatography (HPLC). Positive controls (AFB_1 suspended in the adequate simulated condition) and negative (simulated condition without AFB_1) were included in all the experiments. The tests were carried out in triplicate. The dose selected for AFB_1 was a proven one to reduce the productive performance in production animals (Murugesan et al., 2015).

2.7. In vitro simulated gastrointestinal tract passage

The passage through the simulated GI tract was made according to Armando et al. (2011) with some modifications. The yeast cells (1×10^7 cells/mL) were resuspended in a SS containing AFB_1 (169.3 ± 10.6 ng/mL) and incubated at 38 °C for 5 min under agitation to simulate the chewing process, then the yeast cells were centrifuged at 5000 g for 15 min. After centrifugation, the supernatant containing unbound mycotoxin was collected and stored at −20 °C. The resulting yeast cell pellet was resuspended in the GJS and incubated at 38 °C for 45 min under agitation simulating the peristalsis process. After centrifugation, the supernatant was collected and stored at −20 °C. Finally, the resulting pellet was resuspended in IFS without toxin and incubated at 38 °C for 30 min under agitation. After that, the yeast cells were centrifuged, and the supernatant containing unbound mycotoxin was collected and stored at 20 °C.

All supernatants from each GI tract stage (salivary, gastric and intestinal conditions) were analysed by HPLC.

2.8. Detection and quantification of aflatoxin B_1

Aflatoxin B_1 was quantified by HPLC (Waters e2695; Waters, Milford, MA, USA) with fluorescence detection (Waters 2475 multi λ fluorescence detector) according to Trucksess, Stack, Nesheim, Albert, and Romer (1994) with some modifications. The samples were derivatized with trifluoroacetic acid. Chromatographic separations were performed on a reversed-phase C18 column (150 × 4.6 mm i.d., 5 µm particle size; Phenomenex, Luna, Torrance, CA, USA). The fluorescence of AFB_1 derivatives was recorded at excitation and emission wavelengths of 360 and 460 nm, respectively. The mobile phase (water:-acetonitrile:methanol 4:1:1) was pumped at 1.5 mL/min. The injection volume was 100 µL and the retention time was around 4 min. The detection limit of the technique was 1 ng/mL.

2.9. Statistical analyses

The AFB_1 adsorption under simulated GI tract conditions and the measures of the cell area, diameter, and cell wall thickness were analysed by analysis of variance (ANOVA). Means and standard error (SE) were compared using the Fisher's protected Least Significant Test (LSD) ($p < 0.0001$). The analysis was conducted using Info Stat (version 2.03; University of Cordoba, Argentina) software.

Table 2
Morphometric analysis of the *Saccharomyces cerevisiae* cell wall: area (µm²), diameter (µm) and thickness (µm).

Culture medium	Area (µm²)	Diameter (µm)	Cell wall thickness (µm)
YPD[a]	9.69 ± 0.55 a	3.42 ± 0.13 a	0.08 ± 0.01 b
DDG[b]	6.16 ± 0.55 b	2.84 ± 0.13 b	0.25 ± 0.01 a

[a] YPD: yeast extract-peptone-dextrose.
[b] DDG: Dried distilled grains. Different letters indicate differences, according to Fisher's protected LSD test ($p < 0.0001$).

3. Results

3.1. Ultrastructural analysis of yeast whole cells

Table 2 shows the influence of the culture medium on the yeast cell wall ultrastructure. The morphometric analysis of *S. cerevisiae* RC016 such as area, diameter and the cell wall thickness revealed that both, the area and the cell wall diameter decreased significantly in cells grown in DDG medium (36.4 and 16.9%, respectively) compared to those obtained in YPD medium. However, the cell wall thickness in DDG medium was significantly higher (68%) than that in YPD medium ($p \leq 0.0001$). Fig. 1 qualitatively shows the same behaviour and also the cell wall diameter increased when cell passed through the IFS of both culture media tested.

3.2. Infrared spectroscopy analysis

Fig. 2 shows the IR spectra in the frequency range of 4000–500 cm^{-1} for yeast cells grown in YPD medium, DDG medium and then, subjected to the IFS by FTIR spectrometry. Three main regions corresponding to carbohydrates (500 - 1400 cm^{-1}), proteins (1300 - 1900 cm^{-1}) and lipids (3000 - 3600 cm^{-1}) were observed. The region of carbohydrates included the chemical structure of molecules such as β-glucans (spectral range 900–1400 cm^{-1}) implicated in the AFB_1 adsorption. The spectra of cells subjected to the different treatments demonstrated a similar spectral pattern, indicating a similar chemical composition; however, the absorption intensity of each treatment was slightly different. Cells grown on both culture media showed the highest absorbance when passed through simulated IFS and DDG-grown cells had the highest one. The same behaviour was observed for all the studied macromolecules.

Table 3 summarizes the functional group's assignments of the different bands observed for *S. cerevisiae* growing in different cultural conditions. A semi-quantitative comparison of the main IR bands present in both cell cultures was done. *S. cerevisiae* cells showed similar spectrum when grown at the different cultural conditions. Bands corresponding to –OH stretching and C–H stretching were found in the region between 2900 and 3500 cm^{-1}. The highest absorbance was observed for cells growing in DDG + IFS. The IR bands assigned to amide I and amide II (typical of chitin) were present at 1743 and 1560 cm^{-1}, respectively. Vibration between COC and CC stretching vibrations were also identified in the region between 991 and 1244 cm^{-1}. In addition, in-plane ring deformation of C–H and O–H bending modes appeared in the spectrum (1074-1159 cm^{-1}). β-Linked glycosidic bands were appreciable at ~880 cm^{-1}. In addition, β-(1–4) (1–6)-glucan absorption band was assigned at ~765 cm^{-1}. Bands related with (1–3) (1–6) β-D-glucans appeared between 920 and 1043 cm^{-1} and those present in cells from DDG + IFS had the highest absorbance.

Fig. 3 shows the IR spectra in the frequency range 1450–800 cm^{-1} (polysaccharides profile) for yeast cells grown in YPD medium, DDG medium and then, subjected to the artificial IFS by FTIR spectrometry. Bands of functional groups related to mycotoxins adsorption such as β-D (1–3) (1–6) glucans were analysed and all of them increased in both

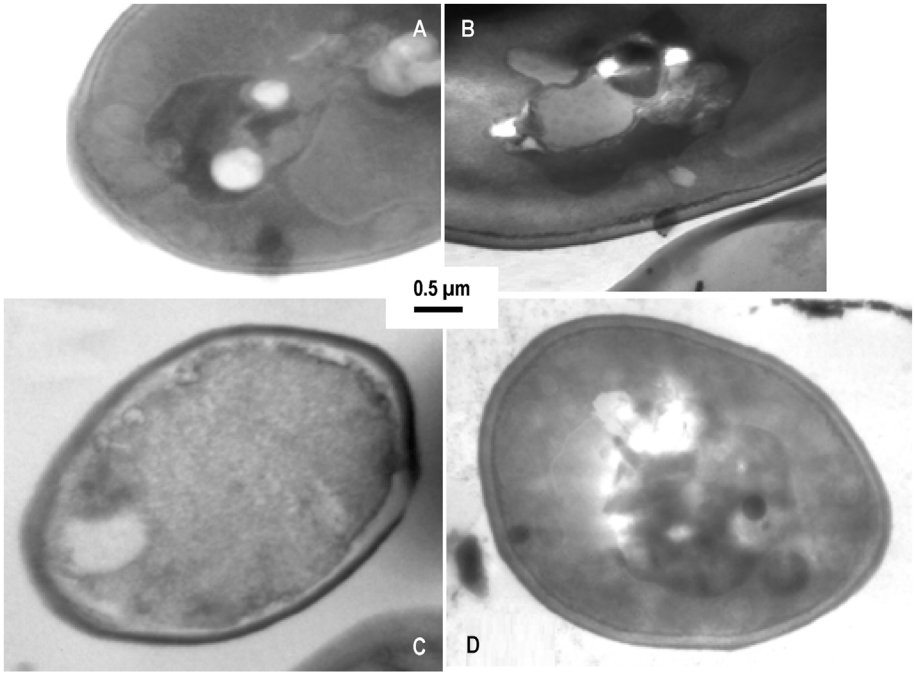

Fig. 1. *Saccharomyces cerevisiae* RC0016 ultrastructure analysis: (A) grown in Dried Distilled Grains (DDG) medium, (B) grown in DDG medium + intestinal fluid solution (IFS), (C) grown in Yeast extract - Peptone - Dextrose (YPD) medium and (D) grown in YPD + IFS. 40000x.

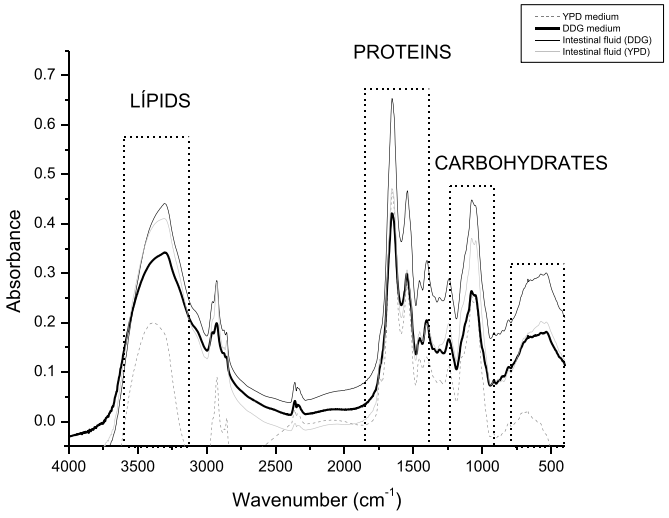

Fig. 2. Macromolecules composition of *Saccharomyces cerevisiae* RC016 grown in different culture media (YPD or DDG) and then, subjected to the intestinal fluid solution by IR spectrometry.

tested media, mainly those in cells grown in DDG medium.

3.3. Effect of simulated gastrointestinal tract on Saccharomyces cerevisiae RC016 ability to bind aflatoxin B_1

When complete GI tract was simulated, the AFB_1 was introduced in SS simulating the first stage of the digestion process when contaminated food is eaten. Considering the transit in the GI tract, the cells Table 4 shows the adsorption data of the AFB_1 present in the simulated GI tract (the initial concentrations present in the SS were 93.9 ± 5.1 ng/mL for YPD medium and 169.3 ± 10.6 ng/mL for DDG medium), after being digested using an *in vitro* method to mimic the conditions of animal GI tract digestion in the presence of the probiotic strain *S. cerevisiae*.

In the control experiment, which consists of each artificial solution of the GI tract without probiotic strain, the AFB_1 recoveries were 105.7%, 97.5% and 88.6% in the SS, GJS and IFS, respectively were in contact with the other solutions (without AFB_1) and then collected to determine the adsorption levels that varied from 22.9% in SS to 81.8% in IFS (when the strain previously grew in YPD medium). When the strain grew in DDG medium, the absorption of AFB_1 varied between 9.2% and 97.7%. In both cases, the highest adsorption occurred at the intestinal level.

4. Discussion

The use of probiotic biological additives with mycotoxin adsorption abilities constitute a promising alternative to minimize exposure to mycotoxins, promote growth and improve the general health of animals, and avoid the use of antibiotic growth promoters that are already prohibited.

The cell wall of microorganisms is composed mainly by polysaccharides, proteins and lipids that offer a series of functional groups (carboxyl, hydroxyl, phosphate and amino groups) as well as hydrophobic adsorption sites (aliphatic carbon chains and aromatic rings) for the possible mycotoxin binding (Ringot et al., 2007). The cell wall of *S. cerevisiae* consists of a β-1,3 glucan network with β-1,6 glucan side chains, which in turn is linked to highly glycosylated mannoproteins that form the outer layer (Yiannikouris et al., 2006; Yiannikouris et al., 2004). The molecular composition and organization of the cell wall can vary considerably and depends largely on the environmental conditions that are greatly affected by external conditions. In the present work, the area, thickness and diameter of the cell wall of *S. cerevisiae* RC016 were statistically evaluated to determine the influence of DDG and a significant increase in the cell wall thickness was observed. Several parameters have been reported, such as the growth medium, growth conditions and growth time, which could influence the properties of the cell wall. Similar to our results, Aguilar-Uscanga and Francois (2003) showed that the cell wall varied in composition and thickness depending on the culture medium composition, temperature, and external pH and oxygen levels. In this sense, Senz, van Lengerich, Bader, and Stahl (2015) demonstrated a direct influence of the culture medium on the cell morphology of a lactic acid bacterium used in the dairy industry as a probiotic supplement and as a starter culture.

Table 3
Functional groups characteristics resulting from curve fitting of the FITR spectra of *Saccharomyces cerevisiae* cell wall produced in medium Yeast extract-Peptone-Dextrose (YPD), in medium Dried Distilled Grains (DDG) and Intestinal Fluid Solution (IFS), simulated.

Functional groups	Wavenumber, cm^{-1} (absorbance)			
	Culture conditions			
	YPD	DDG	IFS (YPD)	IFS (DDG)
-OH stretching	3392 (0.1991)	3303 (0.3421)	3307 (0.4106)	3305 (0.4411)
C–H stretching	2925 (0.0900)	2927 (0.1989)	2939 (0.2370)	2925 (0.2849)
-OH_2 stretching (CH_2 of sugar)	2854 (0.0074)	2856 (0.1310)	2854 (0.1640)	2854 (0.1801)
Amide I (C═O) + (N–H) (indicative of chitin)	1743 (0.0910)	1656 (0.4211)	1651 (0.4705)	1641 (0.6219)
Amide II (N–H) + (C–N) (C═O) (chitin)	1652 (0.4632)	1546 (0.2990)	1546 (0.3069)	1560 (0.4210)
CH_2 in plane (CH_2OH of sugar)	1546 (0.2784)	1454 (0.1687)	1454 (0.1820)	1465 (0.2707)
In plane ring deformation including CH and OH bending modes	1456 (0.1284)	1404 (0.2049) 1352	1402 (0.1985)	1402 (0.3255)
	1406 (0.1308)	(0.1469)	1305 (0.1551)	1311 (0.2509)
	1379 (0.1134)	1311 (0.1453)	1244 (0.1981)	1242 (0.2887)
	1311 (0.0839)	1244 (0.1665)		
	1244 (0.1118)			
(1–3) – α – D - glucan	1367 (0.1017)	1367 (0.1524)	1367 (0.1666)	1367 (0.2641)
COC and CC stretching vibrations (characteristic of	1244 (0.1118)	1242 (0.1665)	1244 (0.1981)	1201 (0.2261)
polysaccharides)	1045 (0.2466)	1078 (0.2638)	1078 (0.3706)	1076 (0.4482)
	1242 (0.1117) 1145	1049 (0.2554)	1047 (0.3658) 1078	991 (0.2561)
	(0.1310)		(0.3706)	
	1078 (0.2551)		1047 (0.3658)	
	1049 (0.2493)		1078 (0.3706)	
			1047 (0.3658)	
Linear structure of β-D-glucan linked through (1–3) linkage	1159 (0.1150) 1074	1159 (0.1508)	1159 (0.2120) 1074	1159 (0.2739)
	(0.2509)	1074 (0.2622)	(0.3692)	1074 (0.4476)
(1–3) (1–6) β-D-glucan	1043 (0.2426)	1043 (0.2499)	1043 (0.3617)	1043 (0.4302)
	702 (0.0207)	916 (0.0832)	914 (0.0858)	929 (0.1705)
	680 (0.0930)	852 (0.0918)	806 (0.1032)	920 (0.1781)
		808 (0.1041)		
β linked glycoside bands	–	–	885 (0.0859)	881 (0.1784)
Referred to anomeric structure around glycosidic	–	–	–	852 (0.1843)
(1–4) (1–6) β-D-glucan	–	765 (0.1200)	765 (0.1138)	769 (0.2105)
(1–3) α-D-glucan characteristic	–	572 (0.1793)	572 (0.2029)	541 (0.2995)
		536 (0.1815)	420 (0.1320)	455 (0.2391)
		420 (0.1234)		420 (0.2240)

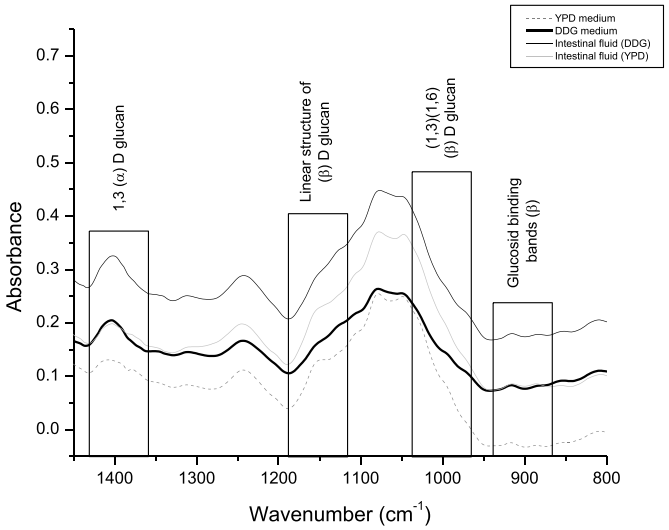

Fig. 3. Polysaccharides profile of *Saccharomyces cerevisiae* RC016 grown in different culture media and then subjected to the intestinal fluid solution by IR spectrometry.

In this work, the adsorption process that simulated the processes of digestion in the mouth (saliva), stomach (gastric conditions) and intestine allowed investigating the adsorption of AFB_1 during the transit through the GI tract and the comparison of adsorption percentages in each portion. The intestine was the stage with the highest percentage of AFB_1 adsorption. These results suggested that the molecular structure of the yeast cell wall was affected since the adsorption process was different at the different stages of the GI tract, probably due to the pH and other components of the tract. In agreement with the present results, Madrigal-Santillán, Madrigal-Bujaidar, Márquez-Márquez, and Reyes (2006) concluded that the adsorption process did not modify the molecular structure of the mycotoxin but also speculated that other factors such as the duration of the incubation, the pH, the method of purification of the biomass and the methods of analysis could also influence this process. Yiannikouris et al. (2004) tested the ZEA adsorption capacity by *S. cerevisiae* at different pH representative of the GI tract and showed that the alkaline conditions seem to impede the active three-dimensional conformation of the β-D glucans and favoured the simple helical structures and/or random spiral. This fact reinforced the importance of evaluating the effect of GI tract on the capacity of *S. cerevisiae* RC016 to adsorb different mycotoxins.

Table 4
Aflatoxin B_1 binding levels (ng mL^{-1}) adsorbed by *Saccharomyces cerevisiae* grown in Yeast extract-Peptone-Dextrose (YPD), Dried Distilled Grains (DDG) media, simulating the gastrointestinal tract passage.

Simulated gastrointestinal tract	AFB_1 binding levels (ng mL^{-1}) (adsorption percentage)	
	Media ± SD*	
	YPD	DDG
Saliva	21.5 (22.9%) ± 6.5 a	13.4 (9.2%) ± 3.5 c
Gastric juice	74.1 (78.9%) ± 0.8 b	121.3 (83.3%) ± 2.1 d
Intestinal fluid	76.9 (81.8%) ± 0.3 b	142.4 (97.7%) ± 0.05 e

YPD: yeast extract-peptone-dextrose; DDG: Dried Distilled Grains. Different letters indicate differences, according to Fisher's protected LSD test ($p < 0.0001$). SD: standard deviation.

The cell wall of the yeast has been shown as an objective for the adsorption of mycotoxins. The present study focused on explaining the adsorption behaviour of AF against the changes in cell wall morphology influenced by the composition of the culture medium. The results showed that the increase in the cell wall thickness achieved more efficient AFB_1 binding cells grown in DDG compared to those grown in YPD. Probably, the pH conditions together the salts and enzymes generate relaxation in the structural structure of the cell wall leaving some functional groups more exposed (β-glucans), which correlates with the increased AFB_1 adsorption capacity. Yiannikouris et al. (2004) showed that the content of β-D-glucan and its three-dimensional arrangement in the cell wall affected the adsorption of mycotoxins. In agreement with our results, Luo et al. (2015) showed that the content of 1,3-β-glucan was well correlated with the cell wall thickness and the capacity to adsorb patulin.

The culture medium DDG is promising for the yeast biomass production that can be exploited as a strategy to develop more effective strains of mycotoxin adsorption. The design and composition of the culture medium should allow the development of the microorganism and maintain its functional and technological properties after scale-up. The optimization of nutritional conditions coupled with the use of spectrophotometric tools could allow to increase in a controlled manner the main components of the cell wall responsible for adsorbing mycotoxins simulating the GI tract. Moreover, the importance of simulating the conditions of each portion of the GI tract (pH, salts, enzymes, temperature and peristaltic movements) to analyse the ability to adsorb AFB_1 was highlighted. Also, an animal study could be carried out to confirm the current results.

5. Conclusion

The use of DDG medium was efficient in producing biomass of *S. cerevisiae* RC016 and exerted valuable modifications in the morphology of the cell wall. The composition of the culture broth was correlated with the cell wall morphology of *S. cerevisiae* and the cell morphology influenced the technological properties such as mycotoxin adsorption. Consequently, the greater the cell wall content, the greater the ability to eliminate AFB_1.

The use of new methodologies such as TEM and IR will allow measuring the variations in the cells morphology and ultrastructure, and the biopolymers profile, respectively: Consequently, the functional properties (probiotic and mycotoxin adsorbents) after scaling the yeast biomass production with low-cost substrates would be controlled, allowing its effective application as a food additive.

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CRediT authorship contribution statement

A.S. Fochesato: Methodology, Validation, Investigation. **A. Cristofolini:** Software, Formal analysis. **V.L. Poloni:** Methodology, Validation, Investigation. **A. Magnoli:** Methodology, Validation, Investigation. **C.I. Merkis:** Software, Formal analysis. **C.A. Dogi:** Software, Formal analysis. **L.R. Cavaglieri:** Conceptualization, Writing - original draft, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.109306.

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