Biomedicina e Farmácia: Aproximações 2

Letícia Bandeira Mascarenhas Lopes
Tiago Sousa Melo
(Organizadores)

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APRESENTAÇÃO

Farmácia e Biomedicina integram o time das ciências da saúde que constituem nas áreas que estudam sobre a vida, a saúde e a doença. No qual focam na manutenção e na melhoria da saúde para o indivíduo, grupos específicos e comunidades.

A obra “Biomedicina e Farmácia: Aproximações” consiste de uma série de livro (E-book) de publicação da Atena Editora, em seus 28 capítulos de artigos científicos do volume I, a qual abordam temáticas atualizadas de diferentes âmbitos que vão desde relatos de casos até a análise de medicamentos, plantas e microbiologia, entre outros.

Sendo assim, almejamos que este livro possa contribuir com informações pertinentes e atualizadas para os estudantes e profissionais da área de farmácia e biomedicina, oportunizando a ampliação dos conhecimentos sobre o tema.

Desejamos a todos uma boa leitura!

Letícia Bandeira Mascarenhas Lopes
Tiago Sousa Melo

SUMÁRIO

CAPÍTULO 18

BIOTECHNOLOGICAL APPLICATIONS OF THE YEAST CELL WALL WITH EMPHASIS ON THE DEVELOPMENT OF FEED ADDITIVES

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ABSTRACT: The objective of the chapter was to give a general look at the applications that yeasts can and could have. Some of them are used as supplements in animal feed due to their relatively high content of proteins and amino acids, energy and micronutrients compared to common cereals and oilseed meals. It has been shown that whole cells, cell walls, wall components improve the performance and health of animal growth. Although, there are many researches that evaluate the use of yeasts in the p otential development and the benefits for human and animal health. It is necessary to develop biotechnological strategies such as the one proposed in this chapter. Four strains of yeasts with probiotic properties (*S. boulardii* RC009; *S. cerevisiae* RC012, *S. cerevisiae* VM014 and *K. marxianus* VM004) were tested to be used as adsorbents of AFB_1. The use of waste from the bioethanol industry was studied as a carbon source to produce biomass and extract the cell wall of the yeasts. The walls were studied using Transmission Electron Microscopy and Fourier Transform Infrared, determining the thickness of the wall and the composition. The quantification of the adsorption of AFB_1 using High Performance Liquid Chromatography was conducted. Increasing the thickness of yeast walls to be used as feed additives and mycotoxin adsorbents is a promising strategy to reduce the exposure to animals (and, consequently, to humans) of mycotoxins, since the capacity of adsorption is due to the interactions between the yeast wall and the mycotoxins.

KEYWORDS: biotechnology, mycotoxin adsorbent, yeast wall cell.

1 I INTRODUCTION

The yeast is a fungus widely used as a model system in basic and applied fields of life science, medicine, and biotechnology. Their primary roles in many food fermentations such as beers, cider, wines, sake, distilled spirits;

bakery products, cheese, sausages, and other fermented foods have been extensively demonstrated. Moreover, they have been also used for the production of fuel ethanol, single cell protein (SCP), feeds and fodder, industrial enzymes, and small molecular weight metabolites. Yeasts are the main producer of biotechnological products in the world, which exceeds the production, capacity and economic income of any other group of industrial microorganisms. The annual world production of *S. cerevisiae* is over 1 million tons (JOHNSON and ECHAVARRI – ERASUN, 2011) (Table 1).

Species	Industrial fermentations	Biotechnological processes	References
Sacharomyces cerevisiae	Beers, Cachaça, ciders, breads, cocoa, wine, silage, fermented meats	Production of proteins and enzymes (pharmaceuticals protein) Invertase (Food applications) L-lactic acid (Biodegradable plastic and textile fibers) Glycerol, Ethanol Vaccines (Medicine)	AMARAL et al., 2008; TAMANG et al., 2009; HONG and NIELSEN, 2012
Schizosaccharomyces pombe	Cachaça,	Heterologous protein	SPENCER et al., 2002
Kluyveromyces lactis and *K. marxianus*	Fermented milks, chesses, dairy products, coffe	Production of enzymes (Chymosin, Lactase) (Food processing) Heterologous protein L-lactic acid (Biodegradable plastic and textile fibers)	ANTONI et al., 2003; RUBIO TEXEIRA 2006; JOHNSON and ECHAVARRI – ERASUN, 2011
Candida spp.	Fermented milks, dairy products, fermented meats	Production of enzymes (lactase, lipases) (food, pharmaceutical and cosmetic industries)	GUO et al., 2006
Pichia spp.	Silage, cocoa	Riboflavin production Heterologous protein	SIBIRNY and BORETSKY (2009)
Debaryomyces hansenii	Chesses, dairy products, fermented meats	Lipid production Carotenoids, surfactants and flavorants	BREUER and HARMS, 2006
Rhodotorula spp.	Fermented meats and sausages, chesses	Production of enzymes (L-phenylalanine) (Industry pharmaceutical) Lipid production	JOHNSON, 2003; AGEITOS et al., 2011
Xanthophyllomyces dendrorhous (*Phaffia rhodozyma*)	Astaxanthin production (Diet animal feed)	Lipid production Astaxanthin production (pharmaceutical industry)	SCHMIDT et al., 2011

Table 1. Yeasts of biotechnological importance and biotechnological products produced.

On the other hand, different yeast species have also been used as prebiotic and probiotic agents for preventing or treating various intestinal, nutritional, and toxicological disorders intended for human health. In recent years, much attention has been paid to the design of functional foods that contain probiotic microbial strains responsible for health benefits in the host (KUMURA et al. 2004).

The main probiotic yeasts are *Saccharomyces boulardii*, *S. cerevisiae* and *K. marxianus* (VIERA et al., 2013; MCFARLAND, 2017; MACCAFERRI et al., 2011).

Some of the properties that make these yeasts as probiotics are the ability to survive the pass through the gastrointestinal tract, help maintain and restore intestinal biota, the non–pathogenicity and the optimal growth at 37°C. Also, they have the ability to antagonise to microbial pathogens (PATIL et al., 2015).

2 I USE OF YEAST IN ANIMAL FEED

For more than 100 years, animals have been fed various forms of yeast and yeast derivatives (STONE, 2006). It has been shown that the use of yeasts provides benefits of animal health and growth performance (GARCIA et al., 2018). Therefore, there are many types of feed additives and feed ingredients that contain yeasts which contribute proteins, vitamins and minerals to the animal diet (Figure 1).

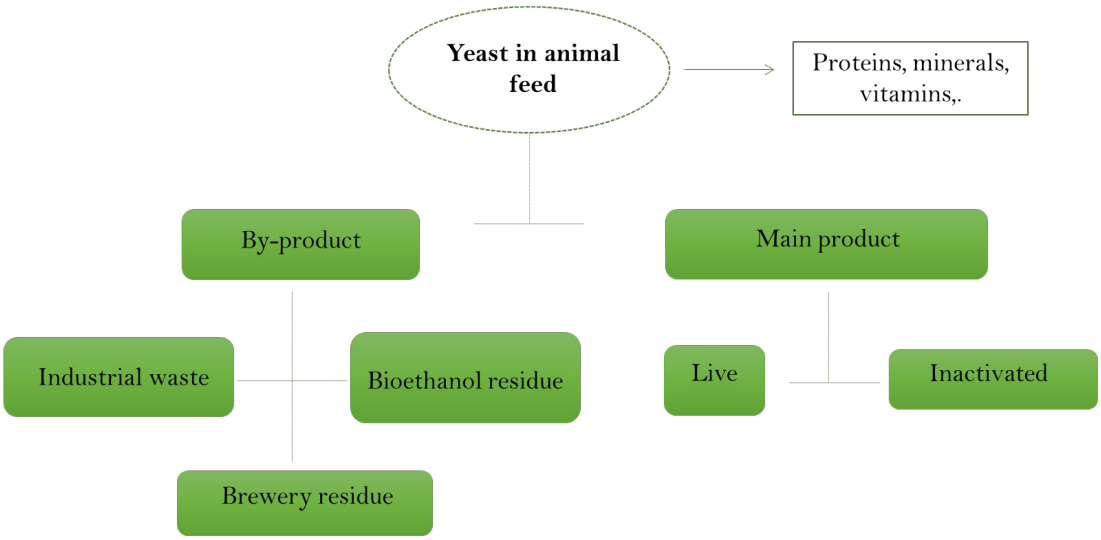

Figure 1. Different contributions of the use of yeasts in animal feed.

Yeast as probiotic and yeast cell wall have been used as adjuncts for animal feeds. The yeast cell wall has been proposed to promote animal growth and health by various mechanisms, including immunomodulation, oxidative status, binding of toxins and pathogens, and interactions with gut constituents. There are several researches that demonstrate the beneficial effects of the live yeast, the yeast walls as well as its components in animal production (Table 2).

Since the prohibition of non-therapeutic use of antibiotics in animal feed by the European Union, the US. The search for new additives that promote growth for animal feed has been increasing in recent years (PATIL et al., 2015).

Species	Cellular part	Benefits	Animal Production	References
Sacharomyces cerevisiae	Whole cell	Probiotic – OTA and ZEN Adsorbent	Pig (*in vitro*)	ARMANDO et al., 2012.
	Whole cell	Probiotic – AFB_1 Adsorbent	Broilers (*in vitro*)	PIZZOLITO et al., 2012.
	Whole cell	Probiotic - AFB_1 Adsorbent	Bovine (*in vitro*)	DOGI et al., 2011.
	YCW	AFB_1 and ZEN adsorbent	Pig (*in vitro*)	PEREYRA et al., 2012.
	Yeast based product and yeast inactivated	AFB_1, ZEN and OTA adsorbent	Animal production (*in vitro*)	JOANNIS CASSAN et al., 2011.
	YCW	OTA adsorbent	Animal production (*in vitro*)	PROTROWSKA and MASEK, 2015.
	YCW and β glucans	AFB_1, ZEN and OTA adsorbent	Animal production (*in vitro*)	YANNIKOURIS et al., 2003; 2004a, 2004b; 2006.
	YCW	Prebiotic - AFB_1 Adsorbent	Broilers (*in vivo*)	BAHAMAN NAVIDSHAD et al., 2015; GUANG – DA XUE et al., 2017; LIU et al., 2018.
	WCY - β glucans	Prebiotic - Immunostimulant	Shrimp (*in vivo*)	SUPHANTHARIA et al., 2003; ACHUPALLAS et al., 2016.
	β glucans	Prebiotic - AFB_1 Adsorbent	Broilers, Pig, Bovine claves	MOON et al., 2016; KERKAERT et al., 2018; NASEER, OMER et al., 2018.
	MOS - Mannan Rich Fraction (MRF)	Immunostimulant	Pig, poultry, Turkey, calves, Aquaculture	CHE et al., 2012; BARRANCO et al., 2014; CHACHER et al., 2017; ROSEN, 2007; MORRISON et al., 2010; RODRÍGUEZ ESTRADA et al. 2013; TORRECILLAS et al., 2011.
	YCW	AFB_1 Adsorbent	Rainbow trout	IMANI et al., 2017.
Candida spp.	Whole cell	Stimulates fermentation	Bovine	MARRERO et al., 2015.
Kluyveromyces marxianus	Whole cell	Probiotic	Pig (*in vitro*)	DÍAZ VERGARA et al., 2017.
Kluyveromyces marxianus	Whole cell	Probiotic	Broilers (*in vivo*)	WANG et al., 2017.
Kluyveromyces fragilis	hydrolyzed or non-hydrolyzed	Immunostimulant	Piglets (*in vivo*)	KEIMER et al., 2018.
Pichia kudriavzevii	Whole cell inactivated	AFB_1 adsorbent	Broilers (*in vivo*)	MAGNOLI et al., 2017.
Ogataea polymorpha	Whole cell	Phytase production (utilization of phosphate)		JOHNSON and ECHAVARRI – ERASUN, 2011.

Table 2. Main yeast species used in animal feed and its benefits.

3 I THE YEAST AS AN ALTERNATIVE TO PREVENT MYCOTOXICOSIS IN ANIMAL PRODUCTION

Mycotoxins are secondary metabolites produced by different species of toxicogenic fungi, such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* genera, which under certain environmental conditions contaminate forages, cereals and different foods (KABAK et.al, 2006). These secondary metabolites comprise a group of more than 400 different chemical compounds, which contaminate the crops before and after harvest, being a common problem throughout the world. In domestic animals, such as dairy cattle, pigs and poultry, mycotoxin contamination reduces growth efficiency, decreases feed conversion and reproduction rates, impairs resistance to infectious diseases, reduces the efficacy of vaccination and induces pathological damage to the liver and other organs (ZHU et.al, 2016). Mycotoxins in general can have different biological and pathological effects: they are capable of triggering acute intoxication, and carcinogenic, mutagenic, teratogenic and estrogenic effects (REDDY et al., 2010).

Aflatoxins are mycotoxins produced by some fungi of the genus *Aspergillus* (*A. flavus* and *A. parasiticus* mainly). Aflatoxin B_1 is considered the most potent natural carcinogen classified by IARC as Group 1 (VILA-DONAT et al., 2018). They can contaminate a wide range of crops such as corn, peanuts, rice, cotton seeds and also animal feed (REDDY et al., 2010; DHANASEKARAN et al., 2011). The contamination of food and feed with this mycotoxin represents great economic losses and generates serious problems in public health due to livestock contamination.

Due to the negative effects that mycotoxins can have on animal and human health, numerous strategies have been developed to prevent or resolve the mycotoxins contamination of food and the fungi that produce them. The mycotoxin adsorbents of biological origin are one of the promissory alternatives to prevent mycotoxicosis in animals. They have the ability to sequester mycotoxins by adsorbing them in the cell wall of bacteria, yeasts and conidia of *Aspergillus* sp. (POLONI et al., 2015, PEREYRA et al., 2016, ZHU et al., 2016). These agents allow the elimination of the mycotoxins found in feed through the faeces, preventing the mycotoxicoses (Figure 2).

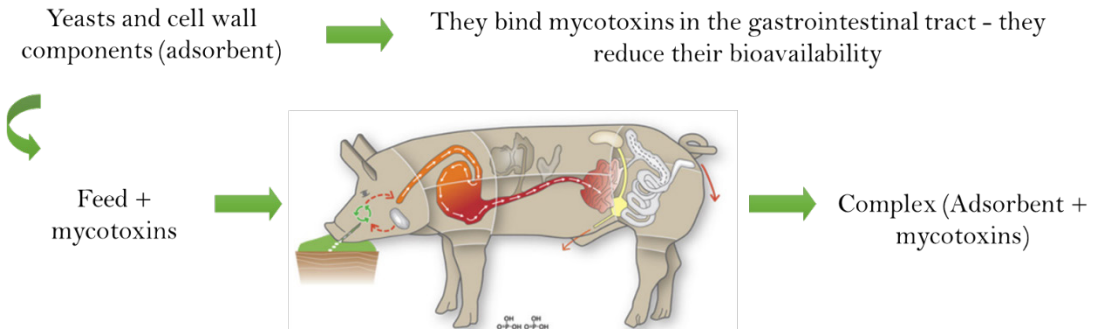

Figure 2. Adsorption of mycotoxins in the gastrointestinal tract using yeast.

4 I YEAST CELL WALL STUDIES

The YCW is an elastic structure that provides osmotic and physical protection and determines the shape of the cell and the integrity of the organism during cell growth and division. The wall has a thickness of about 100-200 nm and comprises 15-20% of the dry weight of the cell (Figure 3). It is composed of three main groups of polysaccharides, mannose polymers (manannoproteins - 40% of the dry weight of the cell), glucose polymers (beta glucans - 60%) and polymers of N-acetylglucosamine (chitin - 2%) (KWIATKOWSKI and KWIATKOWSKI, 2012).

An estimated 1200 genes of *S. cerevisiae* affect the composition and organization of the cell (DE GROOT et al., 2001). It is known that the composition of the wall can vary with respect to different growth conditions, including the type of culture, carbon source, temperature, pH and oxygen availability (PEREYRA et al., 2018).

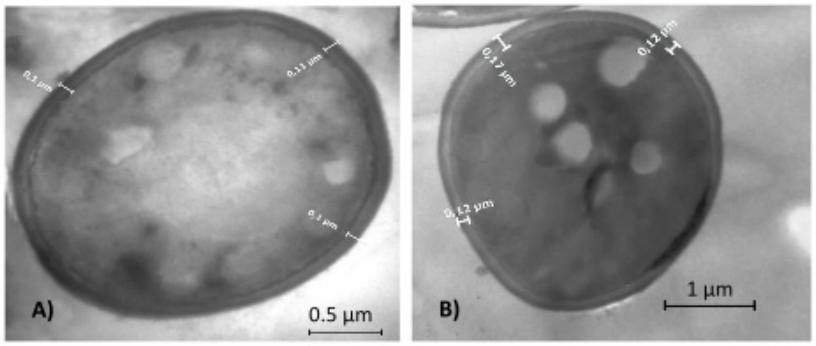

Figure 3. Thickness (µm) of the yeast cell wall of A) *Saccharomyces boulardii* RC009, B) *Kluyveromyces marxianus* VM004.

The cell wall of *S. cerevisiae*, is about 70 nm thickness and represent 20% of the whole cell's weight (WALKER, 1999). Several researchers have studied the variation of the cell wall using different carbon sources and growth conditions (NARUEMON et al., 2013; AGUILAR-USCANGA and FRANÇOIS, 2003). Thus, the alteration of cell wall composition and structure induced by the carbon regimen should be expected. In addition, within each yeast species the strains can act differently under the influence of different nutritional, physical and environmental factors (Table 3).

The importance to study the YCW is based on the fact that the capacity of adsorption between the cell wall and several mycotoxins has already been demonstrated; so, the greater the amount of cell wall, the greater the mycotoxin adsorption capacity. It has a significant economic impact because these microorganisms or only the wall could be used as additive in animal feed to reduce the bioavailability of mycotoxins reducing their toxic effects.

Yeast strains	Culture media	Glucosa (g.L^{-1})	Cell wall production		References
			(g L^{-1})	(%)*	
S. boulardii RC009	YPD	20	1.19	29.6	PEREYRA et al., 2018
	DDGse	2.67	0.22	6.98	
S. cerevisiae RC012	YPD	20	1.85	37	
	DDGse	2.67	0.65	16.2	
S. cerevisiae VM014	YPD	20	0.108	2.22	
	DDGse	2.67	0.761	20.6	
K. marxianus VM004	YPD	20	0.43	8.6	PEREYRA et al., 2017.
	DDGse	2.67	1.41	33.3	
S. cerevisiae		50		29	NGUYEN et al. (1998)
K. marxianus R157		50		29.5	
K. marxianus 1586		50		32.5	
D. hansenii		50		32	
S. cerevisiae		50		22.7	FRANCOIS J. (2006)
S. cerevisiae	YPD	20		25.5	AGUILAR USCANGA and FRANCOIS (2003)
	YNB	-		21.2	
K. marxianus CCEBI 2011		20	3.22	27	SERRAT DÍAZ et al., 2017

Table 3. Influence of the culture medium on the production of biomass and cell wall by different yeast species.

YPD: yeast extract – peptone – dextrose broth. DDGse: dried distiller grains with solubles extract. (*) Percentage of cell wall in relation to the whole cell, based on cell dry weight. YNB: yeast nitrogen base.

5 I INFLUENCE OF AGROINDUSTRIAL WASTE ON THE THICKNESS OF THE CELL WALL AND THE ADSORPTION OF AFLATOXIN B_1

Different substrates have been used for the production of biomass as molasses, starch, cassava, Jerusalem artichoke, whey products, sulphite waste liquor, potato wastes, brewery wastes, and other waste streams from agricultural processes, food processing, and industrial processes (OZYURT and DEVECI 2004).

The most important nutrients for yeasts are carbohydrates that serve for both carbon and energy sources. Mostly hexoses and oligosaccharides, can be fermented by yeasts. 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 from starchy agricultural wastes (SHARMA et al., 2014).

One of the main waste products of the ethanol production industry is the “Distillers dried grains with solubles” (DDGs) commonly used in animal feed as a low-cost supplement that provides energy and proteins. There is a concentration increasement of approximately three times of components such as proteins, fats, vitamins, minerals and fibers. After the conversion of corn starch into ethanol during fermentation.

FOCHESATO et al. (2018) produced a DDGs extract for the biomass production of *S. cerevisiae* RC016 with probiotic properties to be used in animal feed. They

demonstrated that the use of DDGse promotes a sustainable and ecological way to produce yeast biomass. PEREYRA et al. (2018) used the DDGse and a basic medium such as YPD to evaluate the relationship between YCW thickness and cell diameter by transmission electron microscopy (TEM) to determine the proportion of cell wall present in the strain. The relationship showed an accurate estimation of the content of the cell wall (Table 4). The diameter of the cells was similar with the two culture media and the four yeast strains studied. The use of DDGse increased the thickness of *S. cerevisiae* RC012 and *S. cerevisiae* VM014 cell wall. However, there were no differences between the culture media tested with *S. boulardii* RC009 and *K. marxianus* VM004 strains 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 production of biomass giving an added value to the production of cell wall increasing the thickness of the wall used for the mycotoxin adsorption.

Yeast strain	Culture media	Ultrastructural analysis		
		Diameter of whole cell (mm)	Thickness of cell wall (mm)	Cell wall thickness/ cell diameter/ (mm)
S. boulardii RC009	YPD	3.84 ± 0.21	0.126 ± 0.018	0.0328
	DDGse	3.05 ± 0.33	0.095 ± 0.013	0.0311
S. cerevisiae RC012	YPD	3.94 ± 0.74	0.130 ± 0.036	0.0339
	DDGse	3.63 ± 0.37	0.277 ± 0.064	0.0759
S. cerevisiae VM014	YPD	2.31 ± 0.06	0.088 ± 0.013	0.0381
	DDGse	3.27 ± 0.44	0.144 ± 0.023	0.0440
K. marxianus VM004	YPD	2.83 ± 0,21	0.128 ± 0.017	0.045
	DDGse	4.58 ± 0.65	0.167 ± 0.031	0.036

Table 4. Ultrastructural analysis of Saccharomyces boulardii RC009, S. cerevisiae RC012, S. cerevisiae VM014 and K. marxianus VM004: relationship between cell wall thickness/cell diameter (mm).

YPD: yeast extract – peptone – dextrose broth. DDGse: dried distiller grains with solubles extract.

It is important to design an appropriate culture medium depending on the objective, when the biomass or cell wall or some intracellular or extracellular metabolite is required, their production must be optimised in order to reduce production costs making viable the biotechnological process.

6 I EXTRACTION OF THE WALL CELL YEAST AND ITS COMPONENTS

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 et al., 2005; RINGOT et al., 2005).

There are different methods to extract the wall of the yeasts and their components.

The type of extraction and the purity of the component will depend on the use, whether intended for the food, and feed and pharmaceutical or cosmetic industry (NGUYEN et al., 1998; YIANNIKOURIS et al., 2003; SHOKRI et al., 2008; HUANG and LI, 2011; BIN DU et al., 2014; VARELAS et al., 2016).

The composition of the yeast cell wall can be studied using Fourier Transform Infrared (FTIR) spectroscopy that can be applied as a useful tool for the analysis of entire yeast cells providing a fast, effective, reagent-free, and simple method (KULIGOWSKI et al. 2012). The FTIR spectroscopy is a rapid, precise, and accurate method, not requiring sample preparation for the determination and quantification of carbohydrate composition of yeasts (PLATA et al. 2013). In addition, FITR spectroscopy analysis indicated the presence of the C-O, O-H and N-H groups, related to the protein and carbohydrate components, mainly chitin and β glucans involved in the adsorption of AFB_1 (GALICHET et al., 2001).

PEREYRA et al. (2018) studied the spectra the cell yeast walls produced in two culture media (Figure 4) and three (3) regions corresponding to polysaccharides (950 - 1185 cm^{-1}), proteins (1480-1700 cm^{-1}) and lipids (2840 - 3000 cm^{-1}) were observed.

The yeast cell wall spectrum shows three characteristic regions such as carbohydrates, proteins and lipids, which agree with previous works (ADT et al., 2006; AHMAD et al., 2010; NARUEMON et al., 2013; PLATA et al., 2013).

In the same study was observed that the use of YPD increased the amount of total carbohydrates for *S. boulardii* RC009 and *K. marxianus* VM004, 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. GALICHET et al., (2001) obtained spectra similar to ours. They studied the variation of the wall components of a mutated *S. cerevisiae* strain, observing an increase in β glucans and a decrease in mannanoproteins.

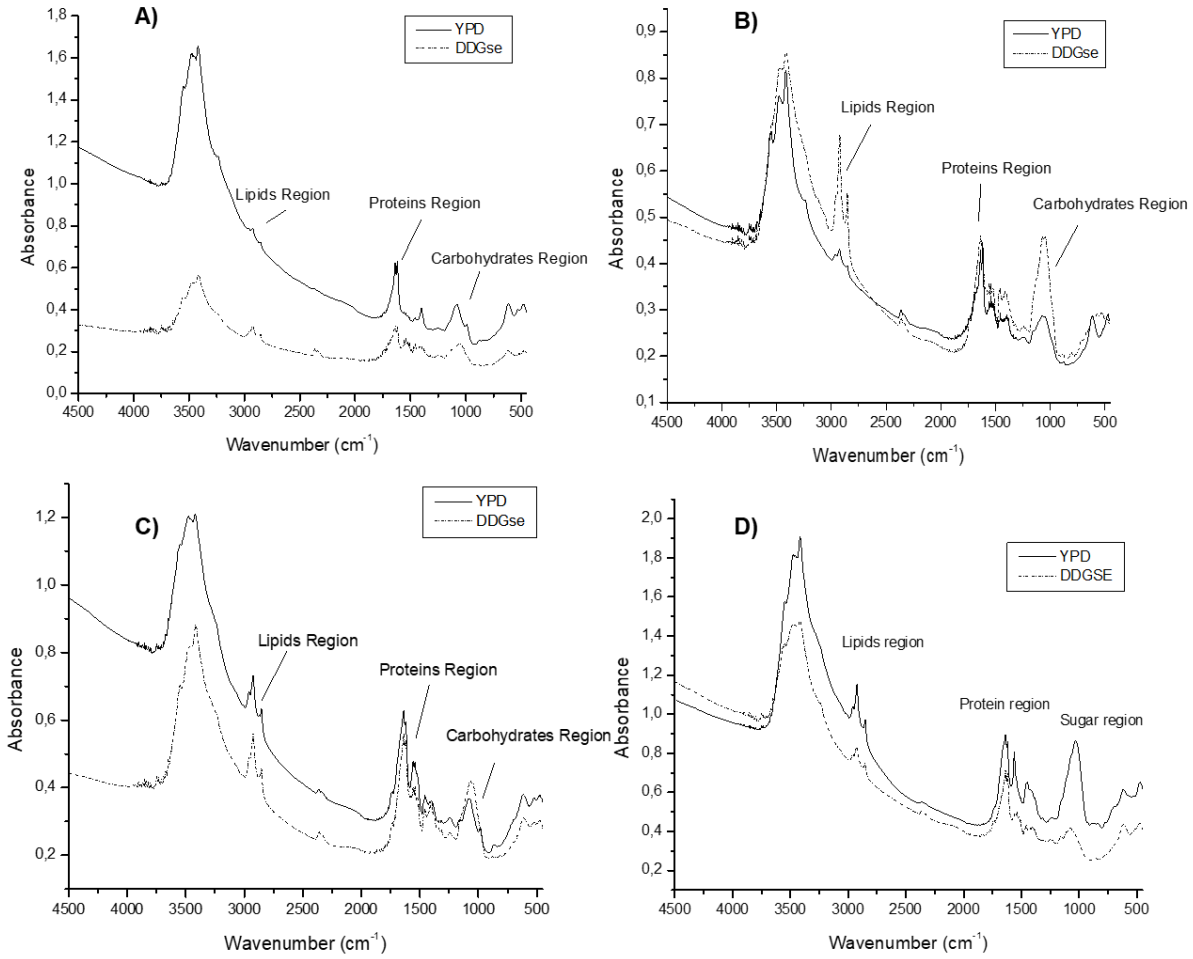

Figure 4. FITR spectra of cell wall of grown in different culture media. A) *Saccharomyces boulardii* (RC009), B) *S. cerevisae* (RC012), C) *S. cerevisae* (VM014), D) *Kluyveromyces marxianus* (VM004).

7 I WALL CELL YEAST AS AN AFLATOXIN B_1 ADSORBENT

The yeast cell walls in particular offers a plethora of possibilities; one of the alternatives is the use as mycotoxin adsorbent.

Several studies have been reported on the biodegradation and adsorption of mycotoxins using different yeast species, mainly *S. cerevisiae* and others such as *Rodhotorula* sp., *Pichia kudriavzevii*, *Clavispora lusitaniae*, *Candida krusei* and *P. anomala*, *C. guilliermondii*, *C. intermedia*, *C. lusitaniae* (ARMANDO et al., 2012; YIANNIKOURIS et al., 2003; YIN et al., 2008; VAR et al., 2009; FIORE et al., 2014; MAGNOLI et al., 2016). In recent years, the use of yeast cell wall has gained importance as adsorbents of mycotoxins, including aflatoxin B_1 (YANNIKOURIS et al., 2003; 2004a, 2004b; 2006).

The potential of the YCW with respect to the whole cell to adsorb AFB_1 has been demonstrated (PEREYRA et al., 2018). Adsorption of the extracted YCW was greater than that using the whole cell (Table 5). However, the use of the whole cell would have a probiotic effect in addition to AFB_1 adsorption. In relation to the yeast cell wall use of some species, they adsorbed almost 10 times more than using the same amount of

whole cell.

There are few studies on the adsorption of AFB_1 using yeast CW (JOANNIS CASSAN et al. 2011). YIANNIKOURIS et al. (2006) found that 6177 μg/mL were adsorbed per 100 μg/mL of CW. PEREYRA et al. (2012) studied the adsorption of AFB_1 with CW of commercial yeasts applying mathematical models to explain the type of interaction of the toxin with the adsorbent that occurred. They found adsorption values of 0.29 ± 0.01 (g/g) at 0.40 ± 0.1 (g/g) for pH 2 and 0.061 ± 0.003 (g/g) at 0.15 ± 0.01 (g/g) at pH 6, showing a relation between the pH and the amount of mycotoxin adsorbed

The use of *S. boulardii* and *K. marxianus* cell walls as AFB_1 adsorbents has not been reported in the literature yet.

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	a	40.47 ± 5.69	b
	DDGse	5.72 ± 0.79		43.82 ± 3.53	
S. cerevisiae RC012	YPD	4.13 ± 1.29		37.49 ± 1.54	
	DDGse	5.01 ± 0.22		37.85 ± 1.76	
S. cerevisiae VM014	YPD	3.43 ± 0.54		35.52 ± 9.28	
	DDGse	4.42 ± 0.40		43.93 ± 3.11	
K. marxianus VM004	YPD	3.69 ± 0.64		48.21 ± 1.09	
	DDGse	6.37 ± 0.09		44.52 ± 1.87	

Table 5. Adsorption of AFB_1 using whole cells and cell wall of *Saccharomyces boulardii* (RC009), *S. cerevisiae* (RC012 and VM014) and *K. marxianus* (VM004) in simulated gastrointestinal pH solution.

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 $P < 0.05$.

It is known that the three-dimensional structure of the polysaccharides constituting the CW allows the adsorption of mycotoxins or their metabolic derivatives (YIANNIKOURIS et al. 2004a, 2004b). DEVEGOWDA and CASTALDO (2000) explained that the interaction of AFB_1 with the CW is from mannan glucans through hydrogen bonds. YIANNIKOURIS et al. (2006) showed that the interaction with mycotoxins is due to the helical conformation of 1-3 β glucans in the complexation of ZEN, AFB_1, DON and PAT. The 1-3 β glucans participate in Van der Waals unions and hydrogen bonds, while 1-6 β glucans strengthen Van der Waals unions and stabilize the interaction.

The efficiency to adsorb mycotoxins is a complex function of the following three factors: chemical structure of the toxin, adsorbent composition and the pH of the medium.

8 I CONCLUSIONS AND CONSIDERATIONS

Yeasts are used as supplements in animal feed due to their relatively high content of proteins and amino acids, energy and micronutrients compared to common cereals and oilseed meals. Whole cells, cell walls, wall components (β-glucans, mannanoligosaccharides) have been shown to improve the performance and health of animal growth. Nowadays, they are commercialized and there are many researches that evaluate the use of yeasts on the potential development and benefits for the health of animals. Still, it is necessary to develop biotechnological strategies such as the one proposed in this chapter. The advances described here demonstrate the potential of cell walls obtained from yeasts isolated from animal environments such as *S. boulardii* RC009 and *S. cerevisiae* RC 0012 and from whey such as *S. cerevisiae* VM014 and *K. marxianus* VM004, all with probiotic properties, to be used as AFB_1adsorbents. In addition, the use of DDGse as a carbon source could replace a synthetic medium as YPD for the production of biomass and CW.

Increasing the thickness of yeast walls to be used as feed additives and mycotoxin adsorbents is a promising strategy. Future studies should optimize biomass production methodologies using different industrial wastes as carbon sources, which is important from the environmental point of view since it would be giving added value to the waste and somehow avoid environmental contamination, optimize wall extraction methodologies and their components to be cost-effective, low-cost products that would help prevent mycotoxicosis in animal production.

In conclusion, the use of these YCW as a mycotoxin adsorbent is a strategy to reduce the exposure to animals (and consequently to humans) of mycotoxins.

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