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Bakery by-products based feeds borne-*Saccharomyces cerevisiae* strains with probiotic and antimycotoxin effects plus antibiotic resistance properties for use in animal production

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ABSTRACT

The aim of this study was to select *S. cerevisiae* strains able to exert probiotic and antimycotoxin effects plus antibiotics resistance properties for use in animal production. *S. cerevisiae* LL74 and *S. cerevisiae* LL83 were isolated from bakery by-products intended for use in animal feed and examined for phenotypic characteristics and nutritional profile. Resistance to antibiotic, tolerance to gastrointestinal conditions, autoaggregation and coaggregation assay, antagonism to animal pathogens and aflatoxin B_1 binding were studied. *S. cerevisiae* LL74 and *S. cerevisiae* LL83 showed resistance to all the antibiotics assayed (ampicillin, streptomycin, neomycin, norfloxacin, penicillin G, sulfonamide and trimethoprim). The analysis showed that exposure time to acid pH had a significant impact onto the viable cell counts onto both yeast strains. Presence of bile 0.5% increased significantly the growth of the both yeast strains. Moreover, they were able to tolerate the simulated gastrointestinal conditions assayed. In general, the coaggregation was positive whereas the autoaggregation capacity was not observed. Both strains were able to adsorb AFB_1. In conclusion, selected *S. cerevisiae* LL74 and *S. cerevisiae* LL83 have potential application to be used as a biological method in animal feed as antibiotic therapy replacement in, reducing the adverse effects of AFB_1 and giving probiotic properties.

1. Introduction

The concept of “functional food”, “animal comfort”, “intestinal health” and “functional supplements” are increasingly present in the current animal production. The base of this concept is that digestibility and a good nutrient absorption are key factors to keep the animal health. These factors depend on a whole intestinal mucosa and healthy intestinal microbiota. The functional supplements are important because of the capacity to keep a healthy microbiota active. The yeast *Saccharomyces cerevisiae* can be used as functional supplement because it provides vitamins and aminoacids, and has the potential use as probiotic (Thornton, 2010).

In the current field of food production, farmers are seeking natural technologies and compounds to improve animal health (Broadway et al., 2014). Fortunately, they are natural alternatives to growth-promoting antibiotics that produce similar effects on performance and overall animal health and well-being (Collier et al., 2010; Eicher et al., 2006). These alternative products are live yeasts and yeast cell wall products derived from *S. cerevisiae* utilized as supplements for performance enhancement and overall benefits to animal health and well-being in the feeding of beef and dairy cattle, swine, lambs and poultry (Beauchemin et al., 2008; Burdick Sanchez et al., 2014; Thrune et al., 2009; Tripathi and Karim, 2011; Van der Peet-Schwering et al., 2007).

Many by-products from the food industry and agriculture are used for the production of food grade yeast biomass and then used

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in animal feed (dairy waste, by-products from the sugar industry, ethanol, fishery and bakery by-products). The use of the various low cost by-products is extremely important, as it provides a solution to the management of these wastes and to the environmental contamination produced by their disposal. These by-products, in addition to providing new alternatives for animal feed, reduce contamination and are used for the production of yeasts that are used in animal feed (Lo Curto and Tripodo, 2001; Suntornsuk, 2000; Zheng et al., 2005).

The pharmaceutical properties and the significant nutritional factors produced by *S. cerevisiae* have merited this yeast to be approved as a Generally Recognized as Safe (GRAS) microorganism for the use in animal feed by the European Union (EEC 70/524), Japanese pharmacopeia (Nitta and Kobayashi, 1999) and Food and Drug Administration (FDA– USA).

Aflatoxins (AFs) are highly toxic secondary metabolites, predominantly produced by some strains of fungi such as *Aspergillus flavus*, *A. nomius*, and *A. parasiticus*. Aflatoxins significantly affect food and feed production because of their detrimental effects on the health of humans and animals (Alberts et al., 2006; Pitt, 2000). The contamination of feed in livestock, with mycotoxins impairs animal health, welfare, and productivity, causing economic important losses (Mellor, 2001). The *S. cerevisiae* capacity of aflatoxins, ochratoxin A (OTA) and zearalenone (ZEA) binding on *in vitro* assays has been widely demonstrated (Armando et al., 2011; Bejaoui et al., 2004; Dogi et al., 2011; Raju and Devegowda, 2000; Shetty et al., 2007; Yiannikouris et al., 2004). Previous studies (Armando et al., 2011; Dogi et al., 2011; González Pereyra et al., 2014; Poloni et al., 2015) have shown that strains of *S. cerevisiae* isolated from healthy animals had the ability to bind aflatoxin B_1 (AFB_1) under gastrointestinal (GI) conditions. Also, these strains have beneficial properties to be considered as probiotic microorganisms. *In vivo* studies were also performed in rats that showed the administration of *S. cerevisiae* RC016 in the diet did not generate genotoxicity or cytotoxicity, improving their potential to be incorporated in the formulation of feed additives.

Farm animals could be affected by several bacterial diseases during the breeding stages, and to combat them the use of antibiotic is necessary, however, currently the world is promoting the replacement of the use of antibiotics due to the problems of bacterial resistance that are present. The animal feed industry is increasingly facing legislative pressures to reduce the use of growth promoters, chemically related to antibiotics. The European Community (EC) prohibits the inclusion of antibiotics as growth promoters (APCs) in feed for poultry and other species of animal origin, requiring new sources of additives which are safe for the Animal and for the human, and on the other hand, that have beneficial effects (Landers et al., 2012). The antibiotic resistance is a desirable feature, the natural resistance of yeast being an important argument for its use as a probiotic (Czerucka et al., 2007).

Although in the literature the joint ability of yeasts to be probiotic and adsorbent of mycotoxins has been reported, no study has shown these properties associated with the replacement of antibiotics. The aim of this study was to select *S. cerevisiae* strains able to exert probiotic and antimycotoxin effects plus antibiotics resistance properties for use in animal production.

2. Materials and methods

2.1. Microorganisms

Saccharomyces cerevisiae LL74 and *S. cerevisiae* LL83 strains were obtained from the Universidade Federal Rural do Rio de Janeiro (UFRRJ), Rio de Janeiro, Brazil collection centre. These strains were previously isolated from bakery by-products intended for use in animal feeds and deposited in this collection centre. The pathogenic bacterial strains obtained from the collection centre of the Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil. These clinical isolates (*Citrobacter sp*., *Serratia marcescens*, *Proteus sp*., *Shigella sp*., *Salmonella sp*. and *Escherichia coli*) were stored in glycerol at −20° C until use. These bacterial strains are associated with clinical infections in animals.

2.2. Morphological and nutritional identification

Yeast strains were examined for phenotypic characteristics (Kurtzman and Fell, 2007; Pitt and Hocking, 2009) and for nutritional profile according to RapID Yeast Plus System ™ (Innovative Diagnostic Systems, Georgia, USA). The RapID Yeast Plus ™ is a system that uses a qualitative micromethod with 18 conventional and chromogenic substrates (Glucose, Maltose, Sucrose, Trehalose, Raffinose, Fatty acid ester, p-Nitrophenyl-N-acetyl- β,D-galactosaminide, p-Nitrophenyl-α,D-glucoside, p-Nitrophenyl-β,D-glucoside, o-Nitrophenyl-β,D-galactoside, p-Nitrophenyl-α,D-galactoside, p-Nitrophenyl-β,D-fucoside, p-Nitrophenyl phosphate, p-Nitrophenyl phosphorylcholine, Urea, Proline-β-naphthylamide, Histidine β-naphthylamide and Leucyl-glycine β-naphthylamide). The reaction was observed and evaluated following the manufacturer's instructions.

2.3. Resistance study of antibiotics frequently used in animal production

The *test* of disc-agar diffusion was based on the description of Mourad and Nour-Eddine (2006) with modifications. Special discs of filter paper impregnated with standardized concentration of antimicrobials (ampicillin 10 μg, streptomycin 10 μg, gentamycin 10 μg, neomycin 30 μg, norfloxacin 10 μg, penicillin G 10 UI, sulfonamide 300 μg and trimetoprim 5 μg (Laborclin®) were placed on Yeast Peptone Dextrose (YPD) agar plate, pre-sowed with a suspension of 10^7 cells/mL of each strain of *S. cerevisiae*. The number of cells/mL was performed using a Neubauer chamber. After incubation for 24 h at 37 °C the inhibition zone was measured in millimetres (mm).

2.4. Beneficial properties of Saccharomyces cerevisiae strains

2.4.1. Tolerance to acid pH

One millilitre of the suspension (10^7 cells/mL) was added to nine (9) mL of YPD broth adjusted to pH 2. The cultures were incubated under constant agitation of 150 rpm at different times 4, 8, 12 and 24 h at 37 °C. Total viable counts of *S. cerevisiae* strains were determined by a plate method using YPD agar after serial 10-fold dilution. Plates were incubated for 24 h at 37 °C.

2.4.2. Tolerance to bile

One mL of the suspension (10^7 cells/mL) was added to 9 mL of broth supplemented with oxgall 0.5% (conjugated bile salts) adjusted to pH 6. The cultures were incubated under constant agitation of 150 rpm at different times 4, 8, 12 and 24 h at 37 °C. Total viable counts of *S. cerevisiae* strains were determined by a plate method using YPD agar after serial 10-fold dilution. Plates were incubated for 24 h at 37 °C.

2.4.3. Viability of yeast strains in simulated gastrointestinal conditions

Simulated gastrointestinal (GI) digestion was tested as described by Fernandez et al. (2003) with some modifications. Briefly, 25 mL of YPD medium were inoculated with each yeast strain (10^7 cells/mL) separately, and incubated with agitation for

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24 h at 37 °C. In order to determine the initial inoculum, an aliquot was taken (T_0) and serial dilutions were carried out and spread on YPD agar. After that, yeast cells were washed in sterile saline solution (NaCl 0.9%) and centrifuged. Cell suspensions were added to 4 mL of artificial salivary secretion (lysozyme 2 mg/mL -Sigma 47700 U/mg in saline solution pH 6.5) and incubated for 5 min at 37 °C (T_1). After that, 8 mL of simulated gastric juice (NaCl 125 mmol; KCl 7 mmol; NaHCO3, 45 mmol and pepsin, 3 g/l; adjusted to pH 3 with HCl) were added. Cells were incubated for 60 min at 37° C under agitation (200 rpm) to simulate peristalsis (T_2). After centrifugation, cells were added to 12 mL of artificial intestinal fluid (trypsin 1 mg/mL-Fluka 11531 U/mg; chymiotrypsin 1 mg/mL-Fluka 80 U/mg; oxgall 0.3% (w/v) in water and adjusted to pH 8 with NaOH 5N) and were incubated for 60 min at 37 °C under agitation (T_3). For the determination of the viable count aliquots were taken of yeast cells (100 μl) after overnight growth and prior to GI passage (T_0) and after salivary (T_1), gastric (T_2) and intestinal (T_3) conditions were taken. Total viable counts of *S. cerevisiae* strains were determined by a plate method using YPD agar after serial 10-fold dilution. Plates were incubated for 48 h at 37 °C.

2.4.4. Autoaggregation assay

Aggregation assay was performed according to Kos et al. (2003) with modifications. Yeasts were grown during 24 h at 37 °C in YPD broth. Cells were harvested by centrifugation and suspended in 0.1% peptone water to Optical Density 1 (OD) units at 600 nm (T_0) measured in a spectrophotometer at 600 nm UV-Vis (FEMTO-Model 600S). Readings were taken at 0, 15, 30, 45 and 60 min incubation at room temperature. The percentage of autoaggregation was determined using the following formula: $1 - (A_t/A_0) \times 100$, where A_t is the absorbance measured at each of the times and A_0 represents the initial absorbance measured at time 0 of incubation.

2.4.5. Coaggregation assay

Yeasts strains were tested for their capacity to coaggregate with bacterial animal pathogens (clinical isolates of *Citrobacter sp.*, *S. marcescens*, *Proteus sp.*, *Shigella sp.*, *Salmonella sp.* and *Escherichia coli*). The inoculum of each pathogen strain was prepared from a 37 °C overnight culture in nutritive broth and harvested by centrifugation. Then, cells were resuspended in PBS (phosphate buffered saline, pH 7).

The assay was performed as was previously reported by Boris et al. (1998) with some modifications. Briefly, 3 mL of each yeast suspension (1×10^7 CFU/mL PBS) was mixed with 3 mL of each pathogen (1×10^7 CFU/mL PBS). Suspension was mixed in a vortex for at least 10 s and then incubated for 4 h at 37 °C, under agitation at 150 rpm. Suspensions were then observed by optic microscopy after Gram staining to evaluate the aggregation degree and scored according to a scale from 0 (no aggregation) to 3 (maximum aggregation).

2.4.6. Antimicrobial capacity against animal pathogens

The antimicrobial capacity of both *S. cerevisiae* strains was performed according to Teo and Tan (2005) with modifications. *S. cerevisiae* strains were grown in YPD broth and incubated for 24 h at 37 °C. Similarly, pathogenic strains *Citrobacter sp.*, *S. marcescens*, *Proteus sp.*, *Shigella sp.*, *Salmonella sp.* and *E. coli* (10^7 CFU/mL) were grown in nutritive broth and incubated for 48 h at 37 °C. A central streak of each yeast strain separately, was performed on Petri dishes containing YPD agar, and incubated for 48 h at 37 °C. Ten mL (10 mL) of additional liquid YPD agar were added to each plate and each pathogenic strain was streaked (perpendicularly) across the same agar plate. After 24 h incubation, antagonistic effect was determined by the appearance of clear zones surrounding the junctions of the streak lines, which indicated the inhibitory effect of one organism on the other.

2.4.7. Mycotoxin binding assay

Yeasts (10^8 cells/mL) were washed twice with PBS and incubated for 1 h at 37 °C in a shaking bath with 1 mL of PBS at each AFB_1 concentration, separately. Then, cells were pelleted by centrifugation for 15 min at 2000 rpm at room temperature, and the supernatant containing unbound mycotoxin was collected and stored at −20 °C for high performance liquid chromatography (HPLC) analysis. Positive (PBS + mycotoxin) and negative (PBS plus yeast cells) controls were included for all experiments. The experiment was conducted in triplicate. For quantification of the unbound AFB_1 used HPLC (Shimadzu® Model HPLC LC-20AT) with UV-Vis detector (model SPD 10A UV) at a wavelength of 360 nm, consisted in a C18 reversed phase column (Betasil, Thermo Scientific®, 250 mm × 10 mm x 5 μm). The mobile phase was isocratic 65% methanol: acetonitrile (90:10) and 35% water with a flow of 1.0 mL/min. The injection volume was 20 μL.

2.5. Statistical analyses

The colony counts data obtained from GI condition analyses and the number of yeast cells were transformed using a logarithmical function $\log_{10}(x + 1)$ before applying the analysis of variance (ANOVA). Means were compared using the Fisher's protected LSD test.

3. Results

3.1. Resistance to antibiotics frequently used in animal production

Fig. 1 shows the effect of antibiotics on growth of both tested *S. cerevisiae* strains. Both yeast showed to be resistant to all the antibiotics tested in this work.

3.2. Study of beneficial properties of Saccharomyces cerevisiae strains

3.2.1. Tolerance to acid pH and bile 0.5%

Table 1 shows the results obtained in the tolerance test of the yeast strains at pH 2 exposed during 24 h. The *S. cerevisiae* LL74 strain did not show a significant difference in counts up to 4 h of exposure, whereas between 8 and 12 h an increase between one and two logarithms was evident, then at 24 h a decrease in the count was observed, maintaining the initial values. For the *S. cerevisiae* LL83 strain, the initial values could be maintained up to 4 h, at 8 h a significant increase in the number of cells occurred and from there, although there is a small decrease in the count, it could be maintained up to 24 h.

In relation to the presence of bile both strains had the same behaviour. The strain *S. cerevisiae* LL74 maintained its initial count until 4 h in the presence of bile, from there a significant increase in the number of cells was obtained, being able to maintain it until the 24 h. *S. cerevisiae* LL83 strain had a similar behaviour and showed a slight increase and remained viable over time.

3.2.2. Viability of the yeast strains in simulated gastrointestinal conditions

The effect of simulated GI transit on the viability of *S. cerevisiae* strains is presented in Table 2. Both yeast strains used in this study preserved viability through salivary conditions (T_1). The passage through the salivary conditions and the GI tract allowed the *S. cerevisiae* strain LL74 a significant recovery, then showed a small decrease in viability after intestinal (T3) passage conditions, whereas *S. cerevisiae* strain LL83 showed a slight decrease in

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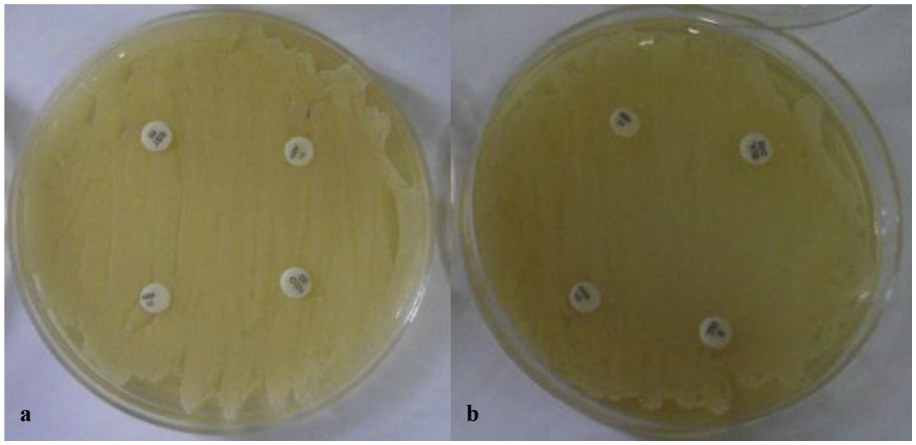

Fig. 1. Strains of *Saccharomyces cerevisiae* resistant to all antibiotics tested. Representative of both strains *Saccharomyces cerevisiae* LL74 and *Saccharomyces cerevisiae* LL83. a) ampicillin (AMP) 10 μg, streptomycin (EST) 10 μg, gentamycin (GEN) 10 μg, neomycin (NEO) 30 μg; b) norfloxacin (NOR) 10 μg, penicillin G (PEN) 10UI, sulfonamide (SUL) 300 μg and trimetoprim (TRI) 5 μg.

Table 1
Total viable counts of *Saccharomyces cerevisiae* strains to pH 2 and bile 0.5%.

Saccharomyces cerevisiae strains	Tolerance assay	Time of exposure (h)				
		0	4	8	12	24
LL74	YPD broth pH 2	7^{bA}	$6.90^{b\ A}$	$8.28^{a\ A}$	$8.44^{a\ A}$	$6.78^{b\ B}$
	YPD broth + bile 0.5% pH 6	$7^{\ cA}$	$7.26^{c\ A}$	$7.64^{b\ A}$	$7.74^{ab\ A}$	$7.84^{a\ A}$
LL83	YPD broth pH 2	$7^{\ bA}$	$6.88^{b\ A}$	$7.65^{a\ A}$	$7.43^{ab\ A}$	$7.28^{ab\ A}$
	YPD broth + bile 0.5% pH 6	$7^{\ bA}$	$7.11^{b\ A}$	$7.52^{a\ A}$	$7.80^{a\ A}$	$7.78^{a\ A}$

Data transformed to $\log_{10}$. Lowercase letters in common in the line are not significantly different according to Fisher's protected LSD test ($P > 0.05$). Uppercase letters in common in the column are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

Table 2
Effect of simulated gastrointestinal (GI) transit on viability of *Saccharomyces cerevisiae* strains.

Saccharomyces cerevisiae strains	Viable cell count (CFU/mL) Simulated GI transit[1,2]			
	T_0	T_1	T_2	T_3
LL74	7.30^{c}	8.55^{a}	8.64^{a}	7.89^{b}
LL83	7.30^{b}	8.48^{a}	8.60^{a}	8.48^{a}

[1]Controls without *S. cerevisiae* strains under each gastrointestinal condition were included.
[2]T0: cell counts prior to assaying the GI transit tolerance; T1: salivary conditions tolerance assay; T2: gastric conditions tolerance assay; T3: intestinal conditions tolerance assay.
Data transformed to log10. Letters in common in the line are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

viability after passage through GI (T_3) conditions, but in general there were no significant differences in counts across the different passages.

3.2.3. Autoaggregation assay

The initial DO (A_0) at 600 nm of both strains (*S. cerevisiae* LL74 and *S. cerevisiae* LL83) was 3000; the same result was obtained at the different incubation times (A_t) for both strains. Therefore, the autoaggregation capacity for both strains was ≤60%.

3.2.4. Coaggregation assay and antimicrobial capacity against animal pathogens

The results obtained from the coaggregation assay and the antimicrobial capacity of *S. cerevisiae* strains against pathogenic bacteria are shown in Table 3. The *S. cerevisiae* LL74 strain was able to coaggregate with all pathogenic bacterial strains tested except with *E. coli*. The degree of coaggregation was greater for *Proteus sp.* and *Shigella sp*. Strains. *S. cerevisiae* LL83 strain was able to coaggregate with all pathogenic bacterial strains tested, obtaining the best results of coaggregation with *Proteus sp.* and *Salmonella sp.* strains (Fig. 2).

Regarding the antimicrobial capacity, both yeasts showed low ability to inhibit the development of the pathogens tested; only *S. cerevisiae* LL74 strain was able to weakly inhibit *S. marcescens* and *Proteus sp.*

3.2.5. Aflatoxin B_1 binding assay

Table 4 shows adsorption capacity of AFB_1 by tested *S. cerevisiae* strains. The highest percentage of adsorption for both strains was observed when tested in the gastric solution. *S. cerevisiae* LL83 strain showed higher adsorption in both solutions compared to strain *S. cerevisiae* LL74.

4. Discussion

The present work describes the analysis of two *S. cerevisiae* strains isolated from bakery by-products intended for use in animal feeds resistant to antibiotics and with probiotic properties added to the ability of binding AFB_1 *in vitro*.

Antibiotic resistance is a desirable characteristic to be found in a probiotic organism, the natural antibiotic resistance of yeast is an important characteristic for their use as probiotics (Czerucka et al., 2007). Furthermore, bacterial resistance can be passing to other bacteria vertical and/or horizontal, through genes transference between bacteria. In the mammal GI tract the conditions are favourable for gene transference among multiple bacteria species. Resistance genes to tetracycline, erythromycin and vancomycin was found and characterized in *Lactobacillus lactis*, *Enterococcus* and,

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Table 3
Co-aggregation and antimicrobial capacity assays of *Saccharomyces cerevisiae* strains against pathogenic bacteria.

Assay	*Saccharomyces cerevisiae* strains	Pathogenic bacteria					
		Citrobacter spp.	*S. marcescens*	*Proteus* spp.	*Shigella* sp.	*Salmonella* sp.	*E. coli*
Coaggregation[a]	LL74	1	2	3	3	2	0
	LL83	2	2	3	1	3	1
Antimicrobial capacity[b]	LL74	−	+	+	−	−	−
	LL83	−	−	−	−	−	−

[a] The score is based upon a scale described by Mastromarino et al. (2002), from 0 for no coaggregation to 3 for maximum aggregation.
[b] +: Inhibition zone ≥3 mm and ≤9 mm; ++: inhibition zone ≥10 and ≤ 15 mm; -: inhibition zone ≤3 mm.

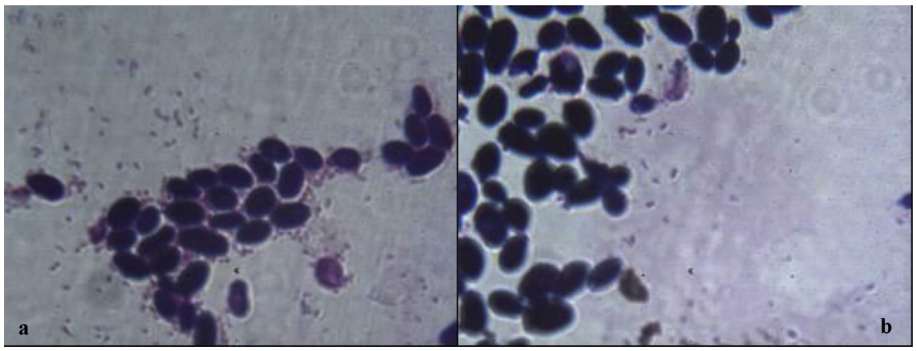

Fig. 2. Coaggregation assay. (a) *Saccharomyces cerevisiae* LL83 and *Salmonella* spp. (+++). (b) *Saccharomyces cerevisiae* LL74 and *E. coli* (-). X400.

Table 4
Capacity adsorption of aflatoxin B_1 by *Saccharomyces cerevisiae* strains.

Strain *Saccharomyces cerevisiae*	%Adsorption Assay conditions GS	IS	AFB_1 (μg/mL)
LL74	30.61	11.46	1.261
LL83	36.00	28.44	

GS: gastric simulated. IS: intestinal simulated.

recently, in species of *Lactobacillus* isolated from meat products and fermented lacteous, and in probiotic strains (Mathur and Singh, 2005; Temmerman et al., 2003). The main threat associated to these bacteria is the capacity of transfer resistance gene to the pathogenic bacteria. Because there is no genetic transference between bacteria and yeast, their use as probiotic is safe and advantageous. In the present work, the two studied yeast strains showed resistance to all the antibiotics tested. The antibiotics affect the pathogenic organism, but also could affect the probiotic strains present on the animal.

A probiotic must have the capacity to survive through the GI tract. Many *in vitro* models consist in simple tests that shown the sensibility of the microorganism to the acid, bile and digestive tract secretions (Marteau et al., 1997) and not susceptibility to antimicrobial therapeutic, frequently used in animal production.

Considering the viability as an important factor, a probiotic must reach a concentration between 10^6 and 10^8 CFU/mL (Charteris et al., 1998). In this study, both studied strains support acid pH, since they had a concentration between 10^6 and 10^7 UFC/mL at the end of the pH tolerance test. Kühle et al. (2004) found that 100% of the *S. cerevisiae* strains tested survived at pH of 2.5 for 4 h. The results observed in this study showed that both strains until the 4 h of incubation maintained the number of cells, and between eight (8) and 12 h they remain their count until the 24 h where the count decreases again. Studies using lactic acid bacteria found that only 0.6% of the *Lactobacillus* and *Bifidobacterium* strains survived at pH 2 for 3 h (Charteris et al., 1997). Also, it was observed that 4.4% of the lactic acid bacteria isolated from goat faeces tolerated the acid conditions (Draksler et al., 2004), and that 16% of the lactic acid bacteria tested as potential swine feed probiotic were capable to resist pH 3 for 3 h (Gusils et al., 2002).

Tolerance to bile salts is considered a prerequisite for colonization and probiotic activity in the small intestine of the host (Havenaar et al., 1992). Both yeast strains were capable of grow at bile 0.3% with probiotic levels, this results agree with Kühle et al. (2004), where 65% of the *S. cerevisiae* tested strains were capable to grow in presence of bile 0.3%. In contrast, the 10% of tested bifidobacteria strains were inhibited in presence of bile, however, when the content of bile was increased to 0.5%, the inhibition was 84% (Liu et al., 2007). Gusils et al. (2002) found that 85% of strains of *L. acidophillus* and *Enterococcus faecium* isolated from porcine faeces were inhibited in the presence of 0.1% bile for 24 h, whereas Draksler et al. (2004) I observed that only 4.5% of lactic acid bacteria used as probiotics for goats could grow in the presence of 0.3% bile.

The ability to tolerate GI passage is generally included among the criteria used to select potential probiotic strains (Morelli, 2000). The two strains of *S. cerevisiae* tested in this study were able to tolerate the passage of secretions from the digestive tract. Different authors reported similar results with strains of *S. cerevisiae* isolated from feces and cheese and strains of *S. cerevisiae var boulardii* isolated from food for humans (Kühle et al., 2004; Psomas et al., 2001). In contrast, other studies showed a much lower level of bacterial recovery, and it was reported that *L. fermentum* isolated from pigs and chickens, decreased 2–3 log when exposed to GI conditions (Gusils et al., 2002; Lin et al., 2007).

The autoaggregation test showed that the studied strains were not able to autoaggregate, contrary to the results found by Armando et al. (2011) who reported yeast that had autoaggregation capacity that varied from 85.3% to 97.9%, while Al-Seraih et al. (2015) showed lower values of self-aggregation (39%) *S. boulardii* strains. Studies performed with LAB showed that 6.6% of *Bifidobacterium*, *Lactobacillus* and *Enterococcus* strains were capable to aggregate (Draksler et al., 2004). In this work, the co-aggregation capacity for both studied strains against all the studied pathogens

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was demonstrated, except *E. coli*. Armando et al. (2011) described that the co-aggregation capacity varied among different strains. Pérez- Sotelo et al. (2005) reported that *E. coli*, *Salmonella typhimurium* and *S. enteritidis* strains bond with *S. cerevisiae* strains; the mechanism of adherence between *Salmonella spp*. strains and yeast was explained by the lecithin type mannose dependent receptors present in the yeast cell wall.

The antimicrobial test showed that only *S. cerevisiae* LL74 had a low antimicrobial activity. This result was in accordance with Draksler et al. (2004) reported that only 0.7% of the *Bifidobacterium sp*., *Lactobacillus sp*. and *Enterococcus sp*. strains tested as probiotics for goats showed antimicrobial activity against *S. typhimurium* and *E. coli*. In contrast, Armando et al. (2011) reported *S. cerevisiae* strains with strong inhibition capacity. In general, few food-borne yeasts have been found to possess clear antagonistic activity (Binetti et al., 2013; Silva et al., 2011).

The utilisation of adsorbents in animal feed to reduce GI tract AFB_1 absorption is one of the most promising and economical strategies (Huwig et al., 2001). In the present work the *S. cerevisiae* LL83 strain reached similar percentages of adsorption both in gastric juice and intestinal solution. However, both strains of *S. cerevisiae* LL74 and LL83 showed higher adsorption capacity when tested in simulated gastric juice (30.61 and 36%, respectively). These results could be given by the pH of the solution as acidic pH favours the attachment of the toxin to the cell, similar results were described by Bovo et al. (2015) where different yeast-based products were tested at pH 3 and 6 with the highest adsorption values being observed for all products tested at pH 3.

In conclusion, the *S. cerevisiae* strains LL74 and LL83 were resistant to the antibiotics frequently used in animal production, tolerate the *in vitro* GI conditions passage, have some probiotic properties, and were capable of binding AFB_1, making them a promising alternative for the development of new additives and probiotic action of mycotoxin decontamination. These strains have potential application to be used as a biological method in animal feed as antibiotic therapy replacement in, reducing the adverse effects of AFB_1 and giving probiotic properties. Future studied should be performed on *in vivo* studies to demonstrate the beneficial properties reported *in vitro*.

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