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R.E.E. Pinheiro
https://orcid.org/0000-0001-5001-9357
A.M.D. Rodrigues
https://orcid.org/0000-0003-0566-9873
C.E. Lima
https://orcid.org/0000-0002-8368-6006
J.T.O. Santos
https://orcid.org/0000-0002-4029-7131
C.M. Pereyra
https://orcid.org/0000-0002-9350-6726
A.M. Torres
https://orcid.org/0000-0002-5452-004X
L.R. Cavaglieri
https://orcid.org/0000-0002-2380-906X
J.B. Lopes
https://orcid.org/0000-0002-0133-4366
M.C.S. Muratori
https://orcid.org/0000-0002-4569-0995

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Saccharomyces cerevisiae as a probiotic agent and a possible aflatoxin B_1 adsorbent in simulated fish intestinal tract conditions

[Saccharomyces cerevisiae *como agente probiótico e possível adsorvente de aflatoxina B_1 em condições simuladas do trato intestinal de peixes*]

R.E.E. *Pinheiro*[1], A.M.D. *Rodrigues*[2], C.E. *Lima*[2], J.T.O. *Santos*[2], C.M. *Pereyra*[3], A.M. *Torres*[3], L.R. *Cavaglieri*[3], J.B. *Lopes*[1], M.C.S. *Muratori*[1]

[1]Universidade Federal do Piauí - Teresina, PI
[2]Aluna de pós-graduação - Universidade Federal do Piauí - Teresina, PI
[3]Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Río Cuarto - Argentina

ABSTRACT

The aim of this study was to evaluate in vitro the probiotic potential and absorption of *Saccharomyces cerevisiae* for the aflatoxin B_1 in simulated fish intestinal tract conditions. Three yeast strains were used, two from brewery: *S. cerevisiae* RC1 and *S. cerevisiae* RC3 and one from a fish farming environment: *S. cerevisiae* A8L2. The selected yeasts were subjected to the following in vitro tests: homologous inhibition, self-aggregation, co-aggregation, antibacterial activity, gastrointestinal conditions tolerance and adsorption of AFB_1. All *S. cerevisiae* strains showed good capability of self-aggregation and co-aggregation with pathogenic bacteria. All yeast strains were able to survive the gastrointestinal conditions. In acidic conditions, the factors (strain vs. time) had interaction (P=0.0317), resulting in significant variation among the strains tested in the time periods analyzed. It was observed that there was also interaction (P=0.0062) in intestinal conditions, with an increased number of cells in the 12-hour period for all strains tested. In the adsorption test, the A8L2 strain was statistically more effective (P<0.005) for both AFB_1 concentrations evaluated in this study (10 and 25ng/mL). Thus, it was observed that the strains of *S. cerevisiae* have potential probiotic and adsorbent of AFB_1.

Keywords**:** adsorption, antibacterial activity, aggregation, homologous inhibition, viability

RESUMO

Objetivou-se, com esta pesquisa, avaliar in vitro *o potencial probiótico e adsorvente de* Saccharomyces cerevisiae *para aflatoxina B_1 em condições simuladas do trato intestinal de peixes. Foram utilizadas três cepas de leveduras, sendo duas provenientes de cervejaria:* S. cerevisiae *RC1 e* S. cerevisiae *RC3, e uma de ambiente de piscicultura:* S. cerevisiae *A8L2. As leveduras selecionadas foram submetidas aos seguintes testes* in vitro*: inibição homóloga, autoagregação, coagregação, atividade antibacteriana, viabilidade às condições gastrointestinais e adsorção de AFB_1. Todas as estirpes de* S. cerevisiae *mostraram boa capacidade de autoagregação e coagregação com bactérias patogênicas. Todas as estirpes de levedura foram capazes de sobreviver às condições gastrointestinais. Em condições ácidas, os fatores (cepa x tempo) tiveram interação (P=0,0317), resultando em variações significativas entre as cepas testadas nos períodos de tempo analisados. Observou-se que também houve interação (P=0,0062) em condições intestinais, havendo um aumento do número de células no período de 12h para todas as cepas avaliadas. No ensaio de adsorção, a estirpe A8L2 foi a mais eficaz estatisticamente (P<0,005), para as duas concentrações de AFB_1 avaliadas neste estudo (10 e 25ng. mL^{-1}). Dessa forma, conclui-se que as cepas de* Saccharomyces cerevisiae *possuem potencial probiótico e adsorvente de AFB_1.*

Palavras-chave: adsorção, atividade antibacteriana, agregação, inibição homóloga, viabilidade

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E-mail: raizza_eveline@hotmail.com

INTRODUCTION

Aflatoxins are toxic metabolites of importance in fishery activities, since their presence has significant negative economic impacts and may cause serious health problems for aquatic species. Among the species diversity, the fish known as tambaquis (*Colossoma macropomum*) stands out for its easy adaptability to different environmental and handling conditions, presenting as a highlight of the global fishing industry. Mycotoxin contamination in aquatic species occurs mainly by ingestion of contaminated feed, as increase in vegetable use in feedstuffs has increasingly caused the presence of these toxic substances in these products (Calvet *et al*., 2015).

Due to the great importance of aflatoxin B_1 (AFB_1) in animal production and its carcinogenic and hepatotoxic potential, the search for adsorbents able to capture these toxins in the gastrointestinal tract and reduce the harmful effects of mycotoxicosis on animal health has increased (Richard and Payne, 2003). The use of physical, chemical and biological control mechanisms is constantly being researched, providing meaningful ways to reduce toxic food contaminants (Richard and Payne, 2003; Pereyra *et al*., 2015).

Among biological methods, microbial detoxification is a promising alternative for reducing mycotoxin levels. Its effectiveness is based on the action of specific chemical compounds produced by a given microorganism. The use of functional foods containing beneficial microorganisms can assist in the mycotoxin decontamination process (Pereyra *et al*., 2015; Armando *et al*., 2011). Probiotic strains formed by Generally Recognized as Safe (GRAS), microorganisms are classified as safe and are one of the most important current biotechnology tools, increasingly becoming a valid alternative in mycotoxin decontamination. *Saccharomyces cerevisiae* and lactic acid-producing bacteria (LAB) have been used in functional foods, probiotics, as well as potential microorganisms for mycotoxin decontamination, due to their AFB_1 binding capability (Pereyra *et al*., 2015; Armando *et al*., 2011; Pizzolitto *et al*., 2012).

According to Juodeikiene *et al*. (2012), *S. cerevisiae* strains with high binding capacity to mycotoxins may be employed as additives in small amounts in many foodstuffs without changing final product characteristics. The inclusion of viable strains in fish diet modifies the intestinal flora by producing antimicrobial substances and enzymes and creating competition with the undesirable microorganisms for adhesion sites and nutrients (Fuller, 1989; Cyrino *et al*., 2010).

For a strain to be considered probiotic, it must meet certain principles, namely: survive the acidic and basic conditions of the gastrointestinal tract; adhere to mucous membranes; proliferate in intestinal conditions, competing with the naturally present microflora; be viable at high concentrations (10^6 to 10^8CFU/mL); not be associated with disease; possess genetic stability during processing and show the absence of undesirable organoleptic food properties (Fuller, 1989; D'Aimmo *et al*., 2007).

The effectiveness of probiotics on animal health has been repeatedly studied. However, besides probiotic capability, it is necessary to evaluate the role of different probiotic strains in the mycotoxin adsorption processes (Armando *et al*., 2011; Pizzolitto *et al*., 2011). In this sense, the searc for natural compounds that can perform such functions is important for technological and economic development and animal health. Thus, in this setting, the present study aimed to evaluate the *in vitro* probiotic and adsorbent potential of *Saccharomyces cerevisiae* regarding aflatoxin B_1 in conditions mimicking fish intestinal tract conditions, for applications in fishery activities.

MATERIAL AND METHODS

Three yeast strains were used, two obtained from a brewery, namely *S. cerevisiae* RC1 and *S. cerevisiae* RC3, and one from a pisciculture environment, *S. cerevisiae* A8L2, obtained from the Food Microbiology Laboratory culture collection at the Food Research and Processing Center (NUEPPA/CCA/UFPI). The selected strains were used for the probiotic testing and AFB_1 adsorption assays. Four strains of pathogenic bacteria provided by the Veterinary Microbiology Laboratory at the Center for Agricultural Sciences (CCA/UFPI) were used for the co-aggregation and antibacterial tests, namely *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and *Pseudomonas* spp.

Selected yeasts were subjected to *in vitro* tests, namely homologous inhibition, self- aggregation, co-aggregation, antibacterial activity and viability to gastrointestinal conditions, to assess their probiotic potential. Subsequently, the yeasts were subjected to an AFB_1 adsorption test. The viability assays were performed in artificially gastrointestinal conditions simulating pH and food transit time conditions in fish known as tambaquis. For this test, three *Saccharomyces cerevisiae* strains were investigated, and the cross-streaking method described by Muzzolón (2010) was applied (Figure 1).

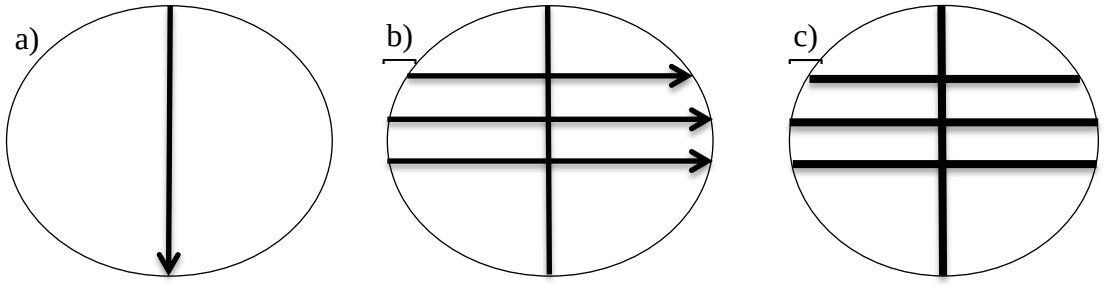

Figure 1. Homologous inhibition assay between *Saccharomyces cerevisiae* strains using cross streaking method a) Vertical streak of tested strains in Petri dishes containing yeast extract peptone dextrose (YPD) agar. b) Horizontal and vertical streaks of tested strains. c) Incubation of Petri dishes at 37°C for 48h.

Self-aggregation capacity assays were carried out according to the methodology described by Kos *et al*. (2003). After a *Saccharomyces* cell count using a double mirrored Neubauer chamber (Herka®) for initial standardization of the inoculate at 10^7cells/mL, the yeast strains were incubated for 24h at 37ºC in 4.0ml of YPD broth. After incubation, the strains were centrifuged at 5.000 rpm for 10 minutes, the supernatants were discarded, and the cells were washed with phosphate buffered saline (PBS) at pH 7.2. The resulting pellets were then resuspended in 4.0mL PBS, pH 7.2, and subjected to homogenization in a vortex shaker.

To assess self-aggregation capacity, the contents were incubated without agitation for two hours at 37ºC in a temperature-controlled culture oven (model 002CB, Fanem LTDA®). Subsequently, 2.0mL of the upper part of the solution were collected and optical density (OD) was measured at 600nm in a spectrophotometer (Model SP - 220, Biospectro). The initial OD was standardized to approximately 0.5. The percentage of self-aggregation was expressed by the following Equation 1: Self-aggregation %=1-(A_t/A_0) x 100, where densities (OD) measured at time zero (A_0) and two hours after the rest time (A_t).

The yeast preparation method for the co-aggregation assays was the same as that used for the self-aggregation assays, following the methodology proposed by Xu *et al*. (2009). Four strains of bacteria associated with infections in fish were used: *Escherichia coli*; *Salmonella* spp., *Staphylococcus aureus* and *Pseudomonas* spp. The inoculate of each pathogenic bacteria were obtained in nutrient broth after 24 hours in a temperature-controlled oven at 37°C. Subsequently, the bacterial suspensions were subjected to centrifugation at 5.000rpm for 10 minutes, the supernatants were discarded and the cells resuspended with PBS, pH 7.2.

Initial standardizations of both the yeast and the bacteria suspensions were carried out in a spectrophotometer at 600nm, to an OD of 0.7, and densities were then adjusted by adding PBS to the more concentrated suspensions. Equal volumes (2.0mL) of the yeast and pathogenic bacteria strains were transferred to a test tube, homogenized in a vortex shaker and incubated at 37°C for two hours without stirring. Finally, the absorbances of the mixtures (OD_{mix}) were measured at 600nm. Co-aggregations were calculated according to the following Equation 2:

$$\text{Co-aggregation } \% = [1 - OD_{mix}/(OD_{yeast} + OD_{bacteria})/2] \times 100$$

The antibacterial activity tests were conducted by the Slab test method on YPD agar in accordance with the methodology proposed by Strus (1998). The pathogenic bacteria strains used in this experiment were *E. coli*, *Salmonella* spp., *S.*

aureus and *Pseudomonas* spp. The bacteria suspensions were standardized with the aid of a spectrophotometer at 600nm to an OD of 0.7. After standardization, the bacteria were plated on nutrient agar plates and incubated at 37°C for 48h. YPD agar pieces of 14mm diameter containing yeasts grown at 37°C after 48h were cut under aseptic conditions and added to the plates containing the bacteria strains. Each treatment was performed in duplicate. Interpretation of the results was performed after 24h incubation at 37°C, by the appearance or non-appearance of clear zones around each strain, indicating the inhibitory effect of a microorganism on the other. The growth inhibition zone diameters around the agar plates were measured and the results given in mm, by subtracting the diameter of the agar plate.

Suspensions of each *S. cerevisiae* isolate strain (RC1, RC3 and A8L2) were prepared in peptone water, with final concentrations of 10^7cells.mL^{-1} using a Neubauer chamber, following the proposal by Van der Aa Kuhle *et al.* (2005) with minor modifications. Subsequently, 900uL of YPD broth was added to 100mL of each solution and the mixture was adjusted to pH 2.0 by addition of hydrochloric acid, to simulate the stomach conditions of the tambaqui (Rotta, 2003). Solutions containing the inoculates were subjected to constant agitation at 150rpm on a shaking table (SL-180/DT model, Solab®) with room temperature maintained at around±30°C. At time points of 4, 8 and 12 hours, 100uL aliquots were obtained from each culture and viable cell count by decimal dilution and seeding by spreading on YPD agar surface. The assay was performed in duplicate.

To determine the viability and tolerance of yeast strains to tambaqui intestine biliary conditions, a test was performed using a methodology similar to the assay regarding tolerance to low pH conditions. YPD broth supplemented with ox bile at 0.5% (Sigma-Aldrich®) was used, adjusted to pH 7.0 by addition of a 1mol/L sodium hydroxide solution, simulating the intestinal conditions of omnivores (Rotta, 2003).

The AFB_1 adsorption assay was performed according to Bueno *et al*. (2007) and Poloni *et al.* (2015) with minor modifications. The initial AFB_1 solution used in the assay was resuspended in acetonitrile from a dry core extract of known concentration (14.7µg AFB_1). Stock AFB_1 solutions (10 and 25ng/mL) were prepared in PBS (pH 2.0 to 7.0). The yeast used in the previously described assays were previously prepared in YPD broth and standardized after growth with the aid of a Neubauer chamber at 10^7cells/mL. Subsequently, 1.0mL of each strain solution was placed into microtubes and centrifuged for 15min at 5.000rpm at room temperature. The samples were then washed with distilled water and subjected to centrifugation in the same conditions. After centrifugation, 1mL of a PBS solution containing AFB_1 was added, initially at pH 2.0, to simulate tambaqui stomach acidity, incubated at 30^{o}C for 30 minutes and then stirred manually. The pellets were centrifuged, and 1.0mL of PBS at pH 7.0 containing AFB_1 at the tested concentrations of 10 and 25ng/mL were added. The microtubes were then incubated at 30°C for 60 minutes and subjected to manual shaking every five minutes, pelleted by centrifugation for 15min at 5.000rpm at room temperature, and the supernatant containing unbound mycotoxins was collected and stored for analysis of the adsorption percentage by high-performance liquid chromatography (HPLC).

AFB_1 detection and quantification was performed by a High Performance Liquid Chromatography (Waters e2695; Waters, Milford, MA, USA) with excitation and emission wavelengths at 360nm and 440nm, respectively, equipped with a C18 reverse phase silica gel column (150 x 4.6mm, 5.0µm particle size, Phenomenex, Luna, Torrance, CA, USA). Aliquots (100uL) of the sample extracts were homogenized in 350uL of a derivatizing solution composed of trifluoroacetic acid:glacial acetic acid:water (20:10:70, v/v) (Trucksess *et al*., 1994). The mobile phase was an isocratic system composed of acetonitrile:methanol:water (17:17:66 v/v) at a flow rate of 1.5mL/min.

The toxin quantification curve was performed by measuring peak heights and performing their interpolation to a calibration curve constructed with different concentrations of the AFB_1 standard, dissolved in acetonitrile, from where the limits of detection and quantification of the technique were extracted. Adsorbed AFB_1 quantifications were established by the correlation between the peak areas of the samples and the standard curve. To calculate the

adsorption percentages, the following Equation 3 was used: Adsorption % = (area of the supernatant / area of the toxin in the positive control) x 100.

The treatments were distributed in a completely randomized 3x4 factorial design (three yeast strains, four viability times) for the viability test and 3x2 (three yeast strains, two AFB_1 concentrations) for the adsorption test, with two replicates per treatment. The colony counts obtained in the gastrointestinal viability test conditions were analyzed and the number of yeast cells transformed on a logarithmic scale $\log_{10}^{(x + 1)}$ and then applied to the analysis of variance. The data obtained (co-aggregation, viability and AFB_1 adsorption) were analyzed according to the SAS 9.0 procedures and subjected to an analysis of variance and mean comparison by Student–Newman–Keuls (SNK) considering a 5% level of significance.

RESULTS AND DISCUSSION

In the homologous inhibition assay, the tested *S. cerevisiae* strains, A8L2, RC1 and RC3, naturally developed between the grooves on the Petri dishes without the presence of a halo between them, as shown in Figure 2.

The results observed herein, therefore, allow for the use of the three strains grouped together, since they did inhibit one another and, if used together, may result in potentiating effects. Such data differ from the earlier report which were conducted by Muzzolón (2010), that demonstrated that 31% of the *Enterococcus* sp. evaluated in inhibited growth of all the LAB strains tested. This homologous inhibition property is important because it allows the use of a mixture of strains to act together as probiotics (De Angelis *et al*., 2006).

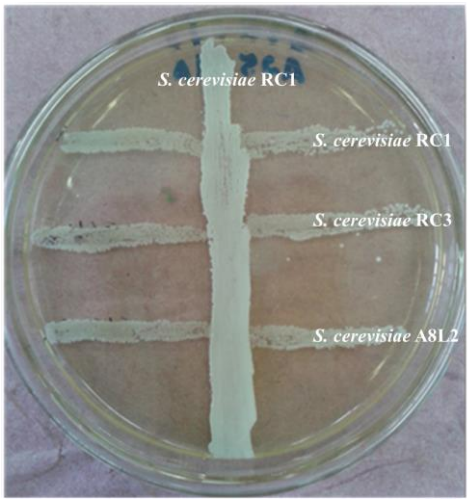

Figure 2. Homologous inhibition between *Saccharomyces cerevisiae* strains. Growth was considered negative when no halo or inhibition (<5mm). A8L2= *S. cerevisiae* obtained from a pisciculture environment; RC1 and RC3= *S. cerevisiae* obtained from a brewery.

With regard to the self-aggregation assay (Table 1) the CR3 strain was more efficient, displaying 87.1% self-aggregation, being considered strong, while strains A8L2 and RC1 showed 76.0% and 78.1%, respectively, although still positive according to the classification proposed by Kos *et al*. (2003).

Table 1. Capacity self-aggregation between *Saccharomyces cerevisiae* strains (means±standard deviation)

Strains	OD_{600} (t_0) [1]	OD_{600} (t_1) [2]	1- (OD_{600} t_1/ OD_{600} t_0) x 100	Score aggregation [3]
A8L2	0.586±0.013	0.140±0.050	76.0±9.10	+
RC1	0.545±0.007	0.119±0.004	78.1±0.62	+
RC3	0.576±0.022	0.074±0.022	87.1±3.33	++

[1]Initial optical density; [2]Optical density after two hours; [3](-) = aggregation ≤ 60, (+) = aggregation< 80 > 60, (++) = aggregation ≥ 80; A8L2 = *S. cerevisiae* obtained from a pisciculture environment; RC1 and RC3 = *S. cerevisiae* obtained from a brewery.

Similar results were reported by Pizzolitto *et al*. (2012) when strains from chicken feed were used, it was found the percentage of 68.4 to 84.7% of self-aggregation. Thus, the self-aggregation percentage observed herein thus indicate that the three investigated strains show good capacity to unite, a desirable characteristic for probiotic species (Muzzolón, 2010). Consequently, the results of homologous inhibition and self-aggregation capacity favor the possible use of these strains grouped the same commercial product formulations.

Although all tested strains are the same species, individual variations (P<0.05) between strains were observed with regard to co-aggregation capacity (Table 2) alongside the pathogenic bacteria used in the test.

For *E. coli*, the A8L2 strain was the most efficient, while for *Salmonella* spp. both A8L2 and RC3 showed higher performance. The RC1 and RC3 strains showed higher percentage of co-aggregation in tests conducted with *S. aureus* and *Pseudomonas* spp. (Table 2). These results were more effective than those obtained by Pizzolitto *et al*. (2012) who found co-aggregation values between 25.3% and 36.0% for *S. cerevisiae* and *S. aureus* strains. In addition, only one of the strains investigated in that study was able to achieve a reasonable level of aggregation (between 15-26%) with the investigated pathogens, of which three were tested in the present study (*E. coli*, *S. aureus* and *Salmonella* spp.).

Table 2. Co-aggregation between *Saccharomyces cerevisiae* and pathogenic strains of bacteria

Strains	Co-aggregation (%)			
	Escherichia coli	*Salmonella* spp.	*Staphylococcus aureus*	*Pseudomonas* spp.
A8L2	50.70[a]	36.14[a]	16.76[b]	49.49[b]
RC1	40.64[c]	30.98[b]	45.29[a]	58.53[a]
RC3	45.71[b]	35.35[a]	42.01[a]	59.52[a]
CV (%)	1.69	2.58	3.39	2.64

Means within the same column followed by different letters differ significantly as per the SNK test (P<0.05). A8L2 = *S. cerevisiae* obtained from a pisciculture environment; RC1 and RC3 = *S. cerevisiae* obtained from a brewery.

For the antibacterial activity test, only the A8L2 strain was able to inhibit *Salmonella* spp., showing an 11mm diameter inhibition, with no significant effect on the other tested strains. The A8L2 strain also presented one of the highest co-aggregation percentages (36.14%) with the bacterium *Salmonella* spp. This is important, since, in order to be considered a probiotic, the strain should be able to inhibit pathogens that occur in the digestive tract of animals, as is the case of *Salmonella* spp. (Fuller, 1989). Keersmaecker *et al*. (2006) in their study also observed the occurrence of antimicrobial activity by a *Lactobacillus rhamnosus* strain against *Salmonella enterica*, while Draksler *et al*. (2004) observed that only 0.7% of the *Bifidobacterium*, *Lactobacillus* and *Enterococcus* strains used as probiotics in food for goats showed antimicrobial activity against *S. typhimurium* and *E. coli*, with the remaining 93.3% being inactive in this regard.

Table 3 and 4 display the survival of *S. cerevisiae* strains in the *in vitro* viability assays regarding simulated tambaqui gastrointestinal conditions. These results of *in vitro* viability assays regarding simulated tambaqui gastrointestinal conditions are essential for the selection of probiotic strains, since these compounds should be tolerant to the presence of stomach acid and bile salts in the intestine (Gueimonde and Salminen, 2006).

All investigated strains showed high survival percentages (Table 3) in the simulated tambaqui stomach pH conditions (pH 2.0). However, both factors (strain vs. time) showed significant interaction (P=0.0317), resulting in significant variations among the tested strains at the analyzed time points. The A8L2 strain proved to be the most stable (P>0.05) from the beginning to the end of the test, while RC1 and RC3 showed variations during the test timeframe. The acidic pH value applied this test may have influenced RC1 and RC3 viabilities between 4h and 8h, with a decrease in cell count during this period (Table 3). Soon after, however, the viable cells became stable, possibly allowing for the multiplication of certain cells in this medium, demonstrated by the increased final count.

Table 3. Effect of the simulated passage at stomach tambaqui pH (pH 2.0) on the viability of *Saccharomyces cerevisiae* strains

Variable	Strains	Time (hours)				Means[1]	CV(%)
		0h	4h	8h	12h		
Viability	A8L2	7.57 ^{aA}	7.56^{abA}	7.74^{aA}	7.56 ^{cA}	7.61	5.03
(log_{10} CFU/mL)	RC1	8.17 ^{aA}	6.84 ^{cB}	7.48^{aB}	7.82^{bAB}	7.58	
(strain/time)	RC3	8.16 ^{aA}	8.16 ^{aA}	6.83^{bB}	8.06 ^{aA}	7.80	
Means[1]		7.97	7.52	7.35	7.81		

[1] Means followed by different lower-case letters within the same column for the same time differ significantly as per the SNK test (P<0.05). Means followed by different capital letters in the same line, for the same strain, differ significantly as per the SNK test (P<0.05). A8L2 = *S. cerevisiae* obtained from a pisciculture environment; RC1 and RC3 = *S. cerevisiae* obtained from a brewery; CFU = colony-forming unit.

Table 4 indicates further interactions between the analyzed factors (P=0.0062). The A8L2 strain proved to be the most effective in these conditions, given that it showed continuous cell growth during all studied time points (4h, 8h and 12h), with a final value of 9.07 CFU/mL. In this sense, Nayak (2010) argues that an ideal probiotic is able to establish, multiply and colonize the intestinal epithelium of the host.

Table 4. Effect of the simulated passage at intestinal tambaqui pH (pH 7.0) on the viability of *Saccharomyces cerevisiae* strains

Variable	Strains	Time (hours)				Means[1]	CV(%)
		0h	4h	8h	12h		
Viability	A8L2	7.65 ^{aD}	7.91^{aC}	8.72^{aB}	9.07^{aA}	8.34	2.64
(log_{10} CFU/mL)	RC1	6.65 ^{bC}	6.53^{bC}	7.27^{bB}	7.85^{cA}	7.08	
(strain/time)	RC3	7.47 ^{aB}	6.30^{bC}	7.57^{bB}	8.52^{bA}	7.47	
Means[1]		7.26	6.91	7.85	8.48		

[1] Means followed by different lower-case letters within the same column for the same time differ significantly as per the SNK test (P<0.05). Means followed by different capital letters in the same line, for the same strain, differ significantly as per the SNK test (P<0.05). A8L2 = *S. cerevisiae* obtained from a pisciculture environment; RC1 and RC3 = *S. cerevisiae* obtained from a brewery; CFU = colony-forming unit.

The tolerance results in simulated tambaqui stomach and intestine conditions are in agreement with those described by Armando *et al*. (2011) who observed that four *S. cerevisiae* strains isolated from food and intestines of pigs were able to survive under simulated gastrointestinal conditions of farm animals. Likewise, in the present study, the A8L2 strain was able to withstand the acidic conditions of the simulated stomach and intestine conditions and showed high concentrations at the end of the assay. Table 5 shows the AFB_1 adsorption capacity for the *S. cerevisiae* strains investigated herein. The yeasts were tested at two different AFB_1 concentrations.

Table 5. Adsorption (%) different levels (10 and 25ng/mL) of aflatoxin B_1 by *Saccharomyces cerevisiae* strains

Variable	Strains	Concentrations (ng/mL)		Means[1]	CV(%)
		10	25		
Adsorption (%) (strains/concentrations)	A8L2	21.19	19.77	20.48^{a}	21.18
	RC1	14.53	4.69	9.61^{b}	
	RC3	13.75	15.20	14.47^{b}	
Means[1]		16.49^{a}	13.22^{a}		

[1] Means followed by different letters differ significantly as per the SNK test (P<0.05). A8L2 = *S. cerevisiae* obtained from a pisciculture environment; RC1 and RC3 = *S. cerevisiae* obtained from a brewery.

No interaction among the factors was found for this variable (P>0.05). The A8L2 strain was statistically more effective (P<0.005) regarding adsorption capacity, of 21.19% and 19.77% adsorption for the two AFB_1 concentrations, respectively, resulting in an overall mean value of 20.48%. Similar values were reported by Pinheiro *et al*. (2017) when simulating the pH of the stomach and intestine of Nile tilapia (*Oreochromis niloticus*) with PBS, who found that the efficiency of the anti-mycotoxin capacity of dry *S. cerevisiae* brewery yeasts at different pH values ranged from 12.4 to 21.6% at 1000ng/mL AFB_1.

Armando *et al*. (2011) using the same yeast cell concentrations as the present study verified that AFB_1adsorption percentages vary between *S. cerevisiae* strains according to the AFB_1 concentration, and found values ranging from 16.4 to 82% for 50ng/mL; 21.3 to 48.7% for 100ng/mL and 20.2 to 65.5% for 500ng/mL AFB_1. However, in contrast to the results presented by those researchers, no difference (P>0.05) was observed between the adsorption values of *S. cerevisiae* strains at 10 and 25ng/mL in the present study. Given these data, it is clear that the amount of adsorbed toxin in different studies is dependent on concentration and binding level, varying between strains, indicating the specific nature of these microorganisms in the adsorption process (Pizzolitto *et al*., 2012). Thus, the best way to evaluate the adsorption efficiency of a microorganism is by using more than one toxin concentration.

The differences in adsorption observed between the three *S. cerevisiae* strains when exposed to different media conditions can be explained by structural differences in yeast cell walls, which are formed by beta-glucans and mananoproteins, specific binding sites for toxins and pathogenic bacteria (Yiannikouris *et al*., 2004; Shety and Jespersen, 2006).

CONCLUSION

Based on the results, the investigated *S. cerevisiae* strains show desirable probiotic characteristics and AFB_1 adsorption capacity. Thus, the inclusion of these strains in tambaqui diets may improve health aspects and reduce the amount of AFB_1 occasionally ingested through contaminated feed. However, *in vivo* studies with these yeast species should be conducted for a more complete evaluation of their use in fish farming.

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REFERENCES

ARMANDO, M.R.; PIZZOLITTO, R.P.; ESCOBAR, F. *et al*. *Saccharomyces cerevisiae* strains from animal environment with in vitro aflatoxin B1 binding ability and anti-pathogenic bacterial influence. *World Mycotoxin J*., v.4, p.59-68, 2011.

BUENO, D.J.; CASALE, C.H.; PIZZOLITTO, R.P. *et al*. Physical adsorption of aflatoxin B1 by lactic acid bacteria and *Saccharomyces cerevisiae*: a theoretical model. *J. Food Prot*., v.70, p.2148-2154, 2007.

CALVET, R.M.; PEREIRA, M.M.G.; COSTA, A.P.R. *et al*. Toxigenic mycobiota and mycotoxins in shrimp feed. *Ciênc. Rural*, v.45, p.1021-1026, 2015.

CYRINO, J.E.P.; BICUDO, A.J.A.; SADO, R.Y. *et al*. A piscicultura e o ambiente: o uso de alimentos ambientalmente corretos em piscicultura. *Rev. Bras. Zootec*., v.39, p.68-87, 2010.

D'AIMMO, M.R.; MODESTO, M.; BIAVATI, B. Antibiotic resistance of lactic acid bacteria and Bifidobacterium spp. isolated from dairy and pharmaceutical products. *Int. J. Food Microbiol*., v.115, p.35-42, 2007.

DE ANGELIS, M.; SIRACUSA, S.; BERLOCO, M. *et al*. Selection of potential probiotic lactobacilli from pig faeces to be used as additives in pelleted feeding. *Res. Microbiol*., v.157, p.792-801, 2006.

DRAKSLER D.; GONZALES, S.; OLIVER, G. Preliminary assays for the development of a probiotic for goats. *Reprod. Nutr. Dev*., v.44, p.397-405, 2004.

FULLER, R. Probiotics in man and animal. *J. Appl. Bacteriol*., v.66, p.365-378, 1989.

GUEIMONDE, M.; SALMINEN, S. New method for selecting and evaluating probiotics. *Dig. Liver Dis*., v.38, p.242-247, 2006.

JUODEIKIENE, G.; BASINSKIENE, L.; BARTKIENE, E. *et al*. Mycotoxin decontamination aspects in food, feed and renewables using fermentation processes. In: EISSA, A.A. (Ed.). *Structure and function of food engineering Rijeka*. Croatia: InTech, 2012. 204p.

KEERSMAECKER, S.C.; VERHOEVEN, T.L.; DESAIR, J. *et al.* Strong antimicrobial activity of *Lactobacillus rhamnosus* GG against *Salmonella typhimurium* is due to accumulation of lactic acid. *FEMS Microbiol. Lett*., v.259, p.89-96, 2006.

KOS, B.; SUSKOVIC, J.; VUKOVIC, S. *et al*. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *J. Appl. Microbiol*., v.94, p.981-987, 2003.

MUZZOLÓN, J. Uso de bacterias lácticas y/o levaduras en la prevención de aflatoxicosis en animales de compañía. 2010. Monografia (Trabajo final para optar al título de Microbióloga) - Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Rio Cuarto, ARG.

NAYAK, S.K. Probiotics and immunity: a fish perspective. *Fish Shellfish Immunol*., v.29, p.2-14, 2010.

PEREYRA, C.M.; CAVAGLIERI, L.R.; KELLER, K.M. *et al*. In vitro ochratoxin A adsorption by commercial yeast cell walls. *Rev. Bras. Med. Vet*., v.37, p.25-28, 2015.

PINHEIRO, R.E.E.; PEREYRA, C.M.; NEVES, J.A. *et al*. In vitro evaluation of aflatoxin B1 adsorption by commercial products used in animal feed. *Arq. Inst. Biol*., v.84, p.1-6, 2017.

PIZZOLITTO, R.P.; ARMANDO, M.R.; COMBINA, M. *et al*. Evaluation of *Saccharomyces cerevisiae* strains as probiotic agent with aflatoxin B_1 adsorption ability for use in poultry feedstuffs. *J. Environ. Sci. Health B.*, v.47, p.933-941, 2012.

PIZZOLITTO, R.P.; BUENO, D.J.; ARMANDO, M.R. *et al*. Binding of aflatoxin B1 to lactic acid bacteria and *Saccharomyces cerevisiae* in vitro: a useful model to determine the most efficient microorganism. In: GUEVARA-GONZALEZ, R.G. (Ed.). *Aflatoxins* – biochemistry and molecular biology. Rijeka, Croatia: InTech, 2011. p.323-346.

POLONI, V.; DOGI, C.; PEREYRA, C.M. *et al*. Potentiation of the effect of a commercial animal feed additive mixed with different probiotic yeast strains on the adsorption of aflatoxin B1. *Food Addit. Contam. A*., v.32, p.970-976, 2015.

RICHARD, J.L.; PAYNE, G.A. (Eds.). *Mycotoxins*: risks in plant, animal, and human systems. Ames: CAST, 2003. 199p. (Task Force report No. 139).

ROTTA, M.A. *Aspectos gerais da fisiologia e estrutura do sistema digestivo dos peixes relacionados à piscicultura*. Corumbá: Embrapa Pantanal, 2003. 48p. (Documentos 53).

SHETY, P.H.; JESPERSEN, L. *Saccharomyces cerevisiae* a lactic acid bacteria as potential mycotoxin decontaminating agents. Review. *Trends Food Sci. Technol*., v.17, p.48-55, 2006.

STRUS, M. A new method for testing antagonistic activity of lactic acid bacteria (LAB) on selected pathogenic indicator bacteria. *Med. Dosw. Mikrobiol*., v.50, p.123-130, 1998.

TRUCKSESS, M.W.; STACK, M.E.; NESHEIM, S. *et al*. Multifunctional column coupled with liquid chromatography for determination of aflatoxins B1, B2, G1, and G2 in corn, almonds, brazil nuts, peanuts, and pistachio nuts: collaborative study. *J. AOAC Int*., v.77, p.1512-1521, 1994.

VAN DER AA KUHLE, A.; SKOVGAARD, K.; JESPERSEN, L. In vitro screening of probiotic properties *of Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. *Int. J. Food Microbiol*., v.101, p.29-39, 2005.

XU, H.; JEONG, H.S.; LEE, H.Y. *et al*. Assessment of cell surfasse properties and adhesion potential of selected probiotic strains. *Lett. Appl. Microbiol*., v.49, p.434-442, 2009.

YIANNIKOURIS, A.; FRANCOIS, J.; POUGHON, L. *et al.* Influence of pH on complexing of model beta-d-glucans with zearalenone. *J. Food Prot*., v.67, p.2741-2746, 2004.