Probiotic evaluation of a craft wheat beer fermented with Saccharomyces cerevisiae UFMG A-905 to treat Salmonella Typhimurium infection in mice

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Abstract

Functional foods containing probiotics are generally administered as dairy products. Non-dairy beverages are another possibility, but probiotic functionality must be confirmed in such vehicles. In the present study, a craft wheat beer brewed with the probiotic yeast *Saccharomyces cerevisiae* UFMG A-905 (905) was evaluated in a murine model of *Salmonella* Typhimurium (ST) infection. Unfiltered or filtered beer brewed with 905, a commercial wheat beer used as a negative control, or saline were administered orally to mice before and during oral ST challenge. High fecal levels of yeast were only counted in mice treated with the unfiltered 905 beer, which also had reduced mortality and body weight loss due to ST infection. Increased levels of intestinal IgA, translocation to liver and spleen, liver and intestinal lesions, pro-inflammatory cytokines in liver and ileum, and hepatic and intestinal myeloperoxidase and eosinophilic peroxidase activities were observed in animals infected with ST. All these parameters were reduced by the treatment with unfiltered 905 beer. In conclusion, the results show that a craft wheat beer brewed with *S. cerevisiae* UFMG A-905 maintained the probiotic properties of this yeast when administered orally to mice challenged with *S.* Typhimurium.

Introduction

Functional foods are those that, in addition to providing basic nutrition, perform other activities that are beneficial to the body. Among foods with functional claims, probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits on the host (1). When offered as functional foods, probiotics are traditionally administered with vehicles based on fermented and/or supplemented milk and its derivatives (yogurt, kefir, ice cream, butter, cheese, acidified milk). However, other matrices for administration of probiotics have already been proposed, such as fruit juices, cookies, breads, sweets, among others (2).

Beer is the third most popular beverage in the world after tea and coffee, and is one of the most preferred alcoholic beverages (3). The search for differentiated products and the appreciation of sensory aspects encourage the production of craft beers, a side of the brewing market that has been growing considerably. In this innovation trend, there are few articles in the scientific literature reporting the production of probiotic beer, such as those in which the yeast *Saccharomyces cerevisiae* var. *boulardii* was used to obtain a functional beverage (4–7). However, in these studies, yeast was not responsible for the fermentation and beer production processes, being only added to the finished beverage. Furthermore, the claim of the beneficial effect of ingesting this probiotic beverage was justified by the presence of antioxidant compounds in beer or by the maintenance of high viable cell counts during storage, without an *in vivo* study. Similarly, a patent was filed by the National University of Singapore for the production of a probiotic sour beer supplemented with *Lactobacillus paracasei* L26 (now *Lacticaseibacillus paracasei* [8]) in which only the viability of lactic acid bacteria in the beverage was determined (9).

Several studies developed at the Departamento de Microbiologia/ICB/UFMG evaluated the probiotic potential of *Saccharomyces cerevisiae* UFMG A-905 in murine models, showing that this yeast colonizes

the digestive tract of germ-free mice at high levels and protects against enteric pathogens (10), reduces the translocation of *Salmonella enterica* serovar Typhimurium and stimulates the immune system (11), preserves the integrity of the intestinal barrier in a model of intestinal obstruction (12), blocks the signals involved in the activation of inflammation in a model of typhoid fever (13), prevents the adhesion of enteropathogens to the intestinal epithelium by co-aggregation *in vitro* and *in vivo* with *S*. Typhimurium, *Salmonella* Typhi and *Escherichia coli* (14), reduces damage caused by inflammatory bowel diseases (15–17), inhibits the allergic symptoms of asthma (18) and protects against food allergy (19).

Based on the information presented above, the yeast *S. cerevisiae* UFMG A-905 was recently used to produce a wheat craft beer with possible functional potential (Know How process entitled: Potentially functional beer production process, No. 202100002 registered on 01/27/ 2021). A sensory test performed with this beer showed that the final product had excellent acceptance (88%) and high purchase intent (87%) by the panel of evaluators. Furthermore, based on a minimum daily consumption of 100 mL, the viable yeast cell content in beer was compatible with its use as a potential probiotic (about 10^7 colony forming units [CFU]/mL). This count was determined after 60 days of storage at room temperature.

The present study aimed to confirm the protective effect offered by *S. cerevisiae* UFMG A-905 against experimental infection by *S*. Typhimurium when administered orally to mice as a live suspension in a craft wheat beer brewed with the yeast itself. Experiments with filtered and unfiltered beer were carried out to evaluate the possible action of extracellular products derived from yeast metabolism.

Material And Methods

Animals

Four-week-old NIH Swiss mice, of both sexes, derived from the conventionalization of germ-free animals, were provided by the Biotério de Gnotobiologia/UFMG (Belo Horizonte, Brazil). The animals were kept in polypropylene microisolators (Alesco, Monte Mor, Brazil), and placed in a ventilated cabinet (Alesco), with lighting (12 h of light, 12 h of darkness), humidity (60–80%) and temperature (22 ± 1°C) controlled. Mice received autoclaved water and commercial diet (Nuvital, Curitiba, Brazil) *ad libitum*.

Microorganisms

The yeast *S. cerevisiae* UFMG A-905 was provided by Prof. Dr. Carlos Augusto Rosa, from the Laboratório de Ecologia e Biotecnologia de Leveduras, Departamento de Microbiologia/ICB/UFMG. The strain was identified as *S. cerevisiae* using the YEASTCOMPARE program (20) and this was confirmed by sequencing the D1/D2 variable domains of large subunits of the rRNA gene. The yeast was maintained in a medium containing 1% yeast extract, 2% peptone and 20% glycerol, and preserved at -80ºC in 2 mL cryogenic tubes. For all experiments, the yeasts were cultivated in YPG medium (1% yeast extract, 2% peptone and 2% glucose) at 37ºC for 24 h, under constant agitation (150 rpm) in a shaker (OrbiCult™ Shaker, Esco, Singapore). Subsequently, the yeast was concentrated by centrifugation to obtain a level of 10^9 CFU/mL.

Salmonella enterica subsp. *enterica* serovar Typhimurium ATCC 14028 used in animal tests is of human origin and belongs to the culture collection of the Departamento de Microbiologia/ICB/UFMG, being kept at -80^{o}C, in brain heart infusion broth (BHI, Difco, Sparks, USA) supplemented with 20% glycerol.

Beer

Wheat craft beer was produced by the Departamento de Engenharia de Alimentos of the Universidade Federal of São João del Rei, Sete Lagoas, Brazil. The product had an alcohol content of 5.23% ABV, bitterness of 8.10 IBU, pH of 4.32 and carbonation of 7.68 vols. A commercial wheat beer (CWB), not known as a probiotic, was used as a negative control.

Treatment

Once bottled, the beers contained about 10^7 CFU/mL of *S. cerevisiae* UFMG A-905 or commercial yeast CWB. Filtered beer (to remove yeast cells) was obtained by passing through a 0.22 µm porosity filter (Merck, Darmstadt, Germany). To simulate human consumption, the animals received by intragastric gavage a daily dose of 0.1 mL of beer, filtered or not, concentrated at 10^9 CFU/mL by centrifugation.

Infection

S. Typhimurium was cultivated in BHI broth (Difco) for 18 h at 37ºC, without agitation. The inoculum of 10^5 CFU of pathogenic bacteria per animal was obtained by successive serial decimal dilutions of the active culture in sterile saline and administered by intragastric gavage (13).

Experimental Design

To determine the evolution of survival and body weight, the animals were divided into the following four groups (12 mice/group): (ST) treated with sterile saline and infected with *Salmonella*; (905FL/ST) treated with UFMG A-905 filtered beer and infected with *Salmonella*; (905UFL/ST) treated with UFMG A-905 unfiltered beer and infected with *Salmonella*; (CWB/ST) treated with CWB unfiltered beer and infected with *Salmonella*. Daily treatment with saline or beer was applied for 10 days before challenge with *S.* Typhimurium ATCC 14028 and maintained until the end of each experiment (28 days after infection).

For determination of fecal yeast count, bacterial translocation, intestinal permeability and evaluation of immunological and histological parameters, the following four groups were used (six to seven animals/group): (CTL) treated only with sterile saline solution; (905) treated only with UFMG A-905 unfiltered beer; (ST) treated with sterile saline and infected with *Salmonella*; and (905/ST) treated with UFMG A-905 unfiltered beer and infected with *Salmonella*. Mice were anesthetized and euthanized on the seventh day after challenge with *S.* Typhimurium to collect samples of blood, liver, spleen and intestinal tissue, and intestinal contents.

Analysis

Fecal Levels of Yeast

To assess fecal yeast levels, immediately before euthanasia, freshly evacuated feces were weighed, homogenized and subjected to serial decimal dilutions in phosphate buffer saline (PBS – 0.4 M NaCl and 10 mM NaH_2PO_4, pH 7.2). One hundred microliters of dilutions 10^{-2}, 10^{-4} and 10^{-6} were plated, in triplicate, on Sabouraud dextrose agar (Difco) supplemented with 100 mg/L of chloramphenicol and incubated at 28ºC for 48 h for yeast counting, which was expressed as $\log_{10}$ CFU/g of feces (10).

Survival and body weight

Survival and body weight data of animals treated or not with beer and challenged with *S.* Typhimurium were recorded until the 28th day after infection. The results were expressed as the percentage of animals that survived the infection and the percentage of ponderal evolution in relation to the initial body weight, respectively (10).

Intestinal Permeability

To determine intestinal permeability, the animals received orally 0.1 mL of diethylenetriaminepentaacetic acid labeled with 15.2 MBq of technetium-99m (^{99m}Tc-DTPA). Four hours after gavage, the animals were anesthetized and 500 µL of blood was collected and placed in appropriate tubes for radioactivity determination using an automatic gamma counter (Wizard 1480, PerkinElmer/Walac, Turku, Finland). Results are presented as the percentage of radiation dose in the blood, which was calculated using the following equation: % dose/g = (cpm of blood/cpm of standard) × 100, where cpm represents the radioactivity counts per min (17).

Translocation

After the mice were euthanized, parts of the liver and spleen were aseptically removed, weighed, macerated in sterile PBS at a 10^{-2} dilution and then subjected to serial decimal dilutions in sterile PBS. One hundred microliters of each dilution were plated on MacConkey agar (Difco), and colonies (only lactose negative) were counted after 24 h incubation at 37°C. Bacterial growth was expressed as $\log_{10}$ CFU/g organ (13).

IgA Determination

The levels of secreted type A immunoglobulins (sIgA) in the intestinal contents of mice were analyzed by the ELISA capture method, in triplicate, as described by Martins et al. (13). The mouse small intestine was removed through cuts at the gastroduodenal and ileocecal junctions, and the intestinal fluid collected, weighed and diluted in the proportion of 500 mg of intestinal content to 2.0 mL of PBS, containing 1% (v/v) protease inhibitor (1 µM aprotinin, 25 µM leupeptin, 1 µM pepstatin, and 1 mM PMSF). The material was centrifuged at 2,000 x *g* for 30 min at 4ºC, the supernatant collected and stored at -20ºC. To measure total sIgA, ELISA plates were coated the day before testing with anti-IgA capture antibody (M-8769, Sigma-Aldrich, Saint Louis, USA). Intestinal fluid samples were diluted 1:100 in PBS-Tween and detection was performed with peroxidase-conjugated anti-mouse IgA antibodies (A-4789, Sigma-Aldrich). Total sIgA concentration was determined using a purified IgA standard (0106-01, Southern Biotechnology,

Birmingham, USA). Readings were taken at 492 nm on a microplate reader (Epoch, BioTek Instruments Inc., Winooski, USA) and the sIgA concentration determined as µg/g of intestinal fluid (12).

Quantification of Neutrophil Accumulation

The extent of neutrophil recruitment in ileum and hepatic tissues was indirectly assessed by measuring myeloperoxidase (MPO) activity. Briefly, tissue was removed and frozen in liquid nitrogen. After thawing, the tissue was homogenized with 1.9 mL of buffer (0.1 M NaCl, 0.02 M NaH_2PO_4, 0.015 M Na_2 EDTA, pH 4.7) and centrifuged at 12,000 *g* for 10 min. The supernatant was discarded and the precipitate subjected to hypotonic lysis. After additional centrifugation, the precipitate was resuspended in 0.05 M NaH_2PO_4 buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide (HTAB; Sigma-Aldrich), frozen three times in liquid nitrogen and centrifuged at 4°C at 12,000 *g* for 10 min. The supernatant was used in an enzymatic assay for MPO activity by measuring the change in absorbance at 450 nm using tetramethylbenzidine (Sigma-Aldrich). Results were presented as arbitrary units in relation to casein-induced MPO activity in murine peritoneal neutrophils and processed in the same way (19).

Quantification of Eosinophil Accumulation

An eosinophilic peroxidase (EPO) assay was performed to indirectly assess the extent of eosinophil recruitment in the ileum and liver. Briefly, 100 mg of tissue was weighed, homogenized in 1.9 mL of PBS and centrifuged at 12,000 *g* for 10 min. The supernatant was discarded and the erythrocytes lysed. The samples were then centrifuged, the supernatant discarded and the pellet suspended in 1.9 mL of 0.5% HTAB in PBS. The samples were frozen three times in liquid nitrogen and centrifuged at 4ºC and 12,000 *g* for 10 min. The supernatant was used in the enzyme assay. Briefly, 10 mg of o-phenylenediamine (OPD) (Sigma-Aldrich) was dissolved in 5.5 mL of distilled water and then 1.5 mL of this OPD solution was added to 8.5 mL of Tris/HCl buffer (pH 8.0), followed by the addition of 7.5 mL of H_2O_2. Using a 96-well plate, 100 µL of substrate solution was added to 50 µL of each sample. After 30 min, the reaction was stopped with 50 mL of 1M H_2SO_4 and the absorbance read at 492 nm. Results were expressed as arbitrary units (19).

Chemokine and Cytokine Determination

The concentrations of the cytokines IL-6, IL-10, INF-γ, TNF, and the chemokine CXCL-1/KC were determined by ELISA in liver and ileum using commercially available antibodies according to the procedures provided by the manufacturer (R&D Systems, Minneapolis, USA). Tissue aliquots (100 mg) were homogenized in 1 mL of PBS containing protease inhibitor (1 µM aprotinin, 25 µM leupeptin, 1 µM pepstatin, and 1 mM PMSF) and 0.05% Tween 20. The samples were then centrifuged for 10 min. at 10,000 rpm, and the supernatant was collected, diluted 1:2 in PBS and used immediately for assays, as previously described (21). Results were expressed as pg/100 mg of tissue.

Histopathology and Morphometry

For histopathological analysis, liver and ileum samples were collected from the animals after euthanasia. The hepatic lobes were longitudinally sectioned followed by fixation in 10% formaldehyde for at least 12

h. Ileum was separated from the mesentery, washed in PBS and extended with the serosa in contact with filter paper. All fecal content was removed without damaging the mucosa and the segments were transferred to Bouin's solution plus 2% glacial acetic acid for 10 min. The pre-fixed ileum was wound in a spiral with the mucosa facing inwards to form a roll from the distal portion to the proximal end, being later submitted to the process of embedding in paraffin, cutting, staining with hematoxylin and eosin (H&E) and mounting the slides. Histological sections of the liver and ileum were coded and examined sequentially by the same pathologist who was unaware of the experimental conditions of each group.

Liver and ileum stained slides were imaged using an Olympus BX51 direct light optical microscope equipped with Image-Pro Express 4.0 software (Media Cybernetics, Bethesda, USA). For the liver, images of the regions most affected by the inflammatory infiltrate were captured using a 20X objective. All captures were made with a resolution of 1392x1040 pixels and transferred via a Cool SNAP-Proof Color video camera (Media Cybernetics) to a video system coupled to a computer. Quantitative cellularity analysis was measured using the ImageJ program (version 1.47f, Wayne Rasband/National Institutes of Health, USA – available online at http://rsbweb.nih.gov/ij/download.html). For this, H&E-stained slides were automatically measured in terms of tissue area and quantified in terms of the number of cell nuclei present per area by scanning the images through the program, thus excluding areas that did not contain tissue. The result was expressed as the number of cell nuclei per analyzed area in micrometers.

Statistical Analysis

The experiments were repeated at least twice, and the parametric variables were submitted to analysis of variance (ANOVA) followed by Tukey's test to compare means. Survival curves were analyzed using the Log-rank test (Mantel-Cox). Statistical analysis and graphing were performed using the GraphPad Prism 5 software (GraphPad Software Inc., San Diego, USA). Values with a significance level lower than at least 5% were considered a statistical difference.

Results

Figure 1 shows that only beer containing viable yeast cells (unfiltered) was able to reduce mortality due to *Salmonella* challenge when compared to the other three groups ($P < 0.01$). Lesser body weight loss was also observed in the unfiltered beer treated group ($P < 0.05$). For this reason, treatments with filtered beer and CWB beer were eliminated in the following steps of the present study.

To assess whether the yeast survived at adequate levels in the gastrointestinal tract to be functional after ingestion, fecal counts were determined (Fig. 2). As expected, yeast levels were very low in the groups that did not receive unfiltered beer (between 2.0 and 3.0 $\log_{10}$ CFU/g of feces), since fungi do not belong to the dominant microbiota of mammals, but can be found at residual levels. On the other hand, yeast counts were very high in the groups treated with unfiltered beer (about 7.5 $\log_{10}$ CFU/g of feces) ($P < 0.0001$).

Experimental infection with *S.* Typhimurium caused an increase in intestinal permeability as observed in the ST group (Fig. 3). This may facilitate the translocation of pathogenic bacteria from the intestinal lumen to the internal compartment via paracellular route. Treatment with unfiltered beer significantly reduced this effect ($P < 0.05$).

As expected from the results described above, high levels of translocation to the liver and spleen were observed in the ST group (Fig. 4). Among the seven mice analyzed in this group, six (86%) had high counts of *Salmonella* in the liver and spleen (from 5.02 to 6.44 $\log_{10}$ CFU/g organ), while only one did not have the pathogenic bacteria in both organs. On the other hand, in the 905/ST group, the presence of *Salmonella* was not observed in both organs.

Figure 5 shows that experimental infection with *S.* Typhimurium induced an increase in sIgA levels in the intestinal fluid of mice, and that this increased production was prevented in animals treated with unfiltered beer ($P < 0.0001$).

During oral *S.* Typhimurium infection, the pathogenic bacteria translocate from the intestinal lumen to the internal compartment and then migrate to the liver, which is the main site of lethal injury. In this organ, the presence and multiplication of *Salmonella* induced an inflammatory process characterized by the recruitment of eosinophilic and neutrophilic cells. This can be indirectly observed by the increase, respectively, of eosinophilic peroxidase (EPO) and myeloperoxidase (MPO) activities in the liver of mice only infected with the pathogenic bacteria (Figs. 6A and B). This increase was avoided by treating with unfiltered beer before infection ($P < 0.0001$). Likewise, an increase in this protective effect was observed in the ileum (Figs. 6C and D).

As expected from the results cited above, *Salmonella* infection induced an increase in the levels of pro-inflammatory chemokine and cytokine in the liver of mice belonging to the ST group, as well as of anti-inflammatory cytokine (Figs. 7A-E). A similar effect was observed in the ileum, but with values around half of those found in the liver (Figs. 7F-J). Treatment with unfiltered beer reduced these levels to baseline in both organs.

In the histopathological analysis of the ileum, six animals were examined per group, and the CTL and 905 groups showed no histopathological alterations (Figs. 8A and B). The ST group presented homogeneous and focal alterations, with an intact muscular intestinal wall with a slight degree of edema of the submucosa and of the villi lamina. A slight increase in goblet cells was observed in intact areas of the surface epithelium and crypts, as well as foci of superficial epithelial erosion associated with increased cellularity in the villi (mononuclear inflammatory cells) and cellular reactional changes in the epithelium (hyperchromasia and mitosis). Very rare intraepithelial lymphocytes (Fig. 8C) and presence of edema and coagulation necrosis (Fig. 8C, insert) were observed. The 905/ST group had an intact intestinal wall with slight villous edema, absence of foci of epithelial erosion, slight increase in cellularity in the villi (mononuclear inflammatory cells) and absence of mitosis (Fig. 8D).

Figure 9 shows representative histological sections of liver from six animals per group with homogeneous changes. The CTL group showed preservation of the hepatic lobular structure without changes in cellularity, with histological aspects within the normal range (Fig. 9A). Group 905 showed preserved lobular and cellular architecture without significant changes, except for a single site with a slight increase in intraparenchymal cellularity in one of the sections shown in Fig. 9B. The ST group presented histological section with loss of hepatic lobular architecture due to intense, predominantly mononuclear inflammatory infiltrate with irregular distribution in the liver parenchyma, sometimes associated with areas of focal hepatic necrosis (Fig. 9C). Histological liver sections from the 905/ST group showed preserved lobular architecture with a slight inflammatory focus, predominantly mononuclear, interrupting hepatic ranks. There was discrete hepatocytic cell atypia (Fig. 9D). These observations were confirmed by the lower liver cellularity and liver weight/body weight ratio in the 905/ST group when compared to the ST group (Figs. 9E and F).

Discussion

After isolation and identification, the initial selection of microorganisms to be used as probiotics is based on *in vitro* and *in vivo* tests to prove some beneficial effects when administered. Subsequently, the biotechnological stage involves the development of a form of administration of the potential probiotic that allows its viability at high population levels during storage and also maintains its beneficial properties. However, the production process and delivery vehicle can change the efficiency of a probiotic (22,23). In this sense, the present study aimed to confirm whether the potential probiotic activity of *S. cerevisiae* UFMG A-905, demonstrated in previous studies (10–19), was maintained when administered as viable cells in a craft wheat beer brewed with this same yeast. S. Typhimurium is ideally suited for studying the interplay between the pathogen, the host, and a probiotic due to the availability of excellent animal models (24). Experimental *S.* Typhimurium infection in a murine model results in a disease that resembles human typhoid fever. Few hours after oral infection, the pathogenic bacterium invades enterocytes, M and dendritic cells in the distal ileum using the type III secretion system (T3SS) encoded by genes belonging to the pathogenicity island I (25). This secretion system consists of two components that allow the pathogen to invade the epithelial lining (T3SS-1) and survive in the host tissue (T3SS-2). Each T3SS injects several dozen proteins, called effectors, into the cytosol of epithelial cells (for T3SS-1) or macrophages (for T3SS-2) to induce bacterial entry or ensure the spread of bacteria in tissue, respectively. After translocation, invasion is characterized by a rapid bacterial multiplication in the liver and spleen, which results in hepato and splenomegaly, respectively. After a week, a plateau phase is established, characterized by the recognition of bacteria by the innate immune system through phagocytic cells. This leads to the production of several pro-inflammatory cytokines (TNF, IL-1, IL-6, IL-12, IFN-γ), as well as to a massive infiltration of monocytes and neutrophils into the sites of inflammation. Bacterial growth in these locations leads to the formation of abscesses containing predominantly polymorphonuclear leukocytes. In these lesions, *S.* Typhimurium resides intracellularly in macrophages. Later, effector mechanisms of adaptive immunity are induced, and anti-*Salmonella* antibody titers increase, in addition to the production of pro-inflammatory cytokines, especially IFN-γ (25). The death of

mice is essentially a result of liver damage triggered by the production of pro-inflammatory cytokines and the induction of nitric oxide synthesis elicited by lipid A. The absence of *S.* Typhimurium translocation observed in mice of the 905/ST group was also described in our previous study (11) and could explain the lower number of liver inflammatory lesions in these animals seven days after challenge with the enteropathogenic bacteria. In the ST group of the present study, the results obtained in the histopathological analysis were consistent with the known kinetics of *Salmonella* invasion. The mild lesions observed in the ileum can be explained by the rapid passage of the pathogenic bacteria through the intestinal epithelium that occurred at the beginning of the invasion, while the more severe damage observed in the liver results from the tropism of the *Salmonella* for this organ after a week of infection, when the first deaths were noticed.

Many mechanisms of action have been proposed to explain how bacterial and yeast probiotics act, such as the production of antagonistic compounds (organic acids, bacteriocins or H_2S), competition for nutrients, inhibition of pathogen adhesion to epithelium (by receptor competition and spatial exclusion, or by adhesion entrapment on the probiotic surface), inhibition of toxin action (by trapping on the probiotic surface, proteolytic degradation of the toxin molecule or its receptor) and modulation of the immune system (stimulation of IgA production or blood clearance, anti-inflammatory capacity during infective or inflammatory pathologies), interference on bacterial-induced signaling pathways, and actions on bacterial virulence factors (26). The production of antagonistic substances to pathogens or anti-inflammatory molecules requires that the probiotic is alive. On the other hand, co-aggregative or immunomodulation actions due to components of the probiotic structure do not depend on the viability. The International Scientific Association for Probiotics and Prebiotics (ISAPP) defined a postbiotic as a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” (27). To meet this definition, postbiotics must contain inactivated microbial cells, cell components, or cell metabolites that induce health benefits. In the present work, the increase in survival to *Salmonella* infection in animals treated with unfiltered beer (from 33–75% in the ST and 905UFL/ST groups, respectively) was similar to that observed in the initial selection of *S. cerevisiae* UFMG A-905 as a potential probiotic (from 15–55% in the ST and 905/ST groups, respectively) (10). These results may be due to an action of probiotic yeast cells and/or their postbiotic extracellular metabolites. As this protective effect was not observed in filtered beer (905FL/ST), this seems to eliminate the second hypothesis. However, the *in situ* production of active metabolite in the intestine after ingestion cannot be ruled out. Indeed, the culture supernatant of *Saccharomyces boulardii* exhibits an anti-inflammatory effect by interfering with *Salmonella*-stimulated cell signaling pathways, suggesting that a soluble factor produced by yeast is implicated. This supernatant contains a small (1 kDa) heat-stable, water-soluble anti-inflammatory molecule that inhibited the activation of NF-κB by LPS, IL-1β and TNF (28).

sIgA, the dominant antibody isotype in the intestinal mucosa, is produced locally by activated B lymphocytes in the lamina propria. Activation of B cells for the production of IgA can occur either dependently or independently of T lymphocytes. In mice, most of the IgA is produced by T cell-independent mechanisms, contrary to what happens in humans. sIgA prevents bacterial access to the

apical surface of epithelial cells, trapping bacteria in the mucus layer, which plays a crucial role in preventing the invasion of pathogenic microorganisms (29). In the present study, the reduction of sIgA levels in the intestinal fluid of mice treated with unfiltered beer when compared to the ST group may be due to the lower intestinal population of *Salmonella* through greater elimination of pathogenic bacteria by entrapment on the yeast surface as demonstrated previously (14).

Rodents lack a direct homologue of IL-8, but the chemokine CXCL-1/KC is regarded as functional homologue of IL-8. Chemokine ligand 1 (CXCL-1/KC) is a small peptide belonging to the CXC chemokine family that acts as a chemoattractant for various immune cells, mainly neutrophils, to the site of injury or infection and plays an important role in the inflammatory response. The simultaneous increases in CXCL-1/KC levels and MPO activity in the ST group are therefore coherent. As an anti-inflammatory cytokine, IL-10 serves to antagonize the pro-inflammatory effects of other cytokines and can thus keep inflammation in check. In the present study, the increase in its production, accompanying that of pro-inflammatory cytokines, was therefore expected.

As described in the study of Tiago et al. (14), electronic microscopy showed that *S.* Typhimurium cells bound preferentially to *S. cerevisiae* UFMG A-905 than to intestinal epithelial cells when the yeast was present. This may be a mechanism by which this probiotic yeast prevents the adhesion of pathogens to specific receptors in the intestinal epithelium and subsequent host invasion. The resulting lower load of adherent bacteria could explain the lower translocation to the spleen and liver and, consequently, the reduction of pro-inflammatory cytokines and chemokines and the lower recruitment of neutrophils and eosinophils in mice treated with unfiltered beer when compared with the group ST.

Before ending this discussion, it is important to remember that excessive beer consumption has deleterious effects on the human body, with an increased risk of diseases and important social problems such as addiction, accidents and violence. However, some data show that a moderate consumption of beer does not produce the main known chronic damage, and that even some benefits against cardiovascular diseases are observed (30). Recently, the cardiovascular health effects of alcohol have been classified as having a J-shaped curve, in which consumers with low to moderate intake are less at risk than non-drinkers throughout life, whereas those who drink exceedingly have the highest risk (31). It should also be remembered that beer contains, in addition to ethanol, vitamins, phenolic compounds, bitter components of hops, essential oils and biogenic amines (32). In addition, its moderate consumption appears to have positive effects for the body, as it increases the cholesterol associated with high density lipoproteins (HDL), reducing the risks of diseases and cardiovascular accidents (33–35). Finally, recent studies showed that changes observed in a few microbial taxa, and the higher butyric acid concentration in intestinal contents of consumers versus non-consumers of beer, suggest a potentially beneficial effect of moderate beer consumption on gut microbiota and intestinal health (36,37). Despite these properties, we must remember that beer like any alcoholic beverage should be consumed in moderation.

Conclusion

In conclusion, the results obtained in the present study show that a craft wheat beer fermented by *S. cerevisiae* UFMG A-905 maintained the probiotic properties of this yeast when administered orally to mice challenged with *S.* Typhimurium. The protection offered by this treatment was apparently due to the cells, and not to some extracellular metabolite that would be produced by the yeast.

Declarations

Author Contribution

SRMO, APTU, FSM and JRN conceived and designed the experiments; SRMO, LLC, MNSA, BG, ROS, MFR, KDV, SOAF, MSJ and AMS performed the experiments; SRMO, APTU, MFR, KDV, SOAF, MSJ, AMS, FSM and JRN analyzed the data; VNC and SOAF contributed data or analysis tools; and JRN wrote the paper.

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Data Availability

The datasets generated during the current study are available from the current author on request.

Research Involving Human and Animal Participants

All experimental procedures were performed in accordance with the norms of the Brazilian Society of Animal Science/Brazilian College of Animal Experimentation (available at http://www.mctic.gov.br/concea) and approved by the Ethics Committee in Animal Experimentation of the Universidade Federal de Minas Gerais (Protocol Nº 196/2019, CEUA/UFMG).

Conflict of Interest

The authors declare that they have no conflict of interest.

Disclaimer

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Figures

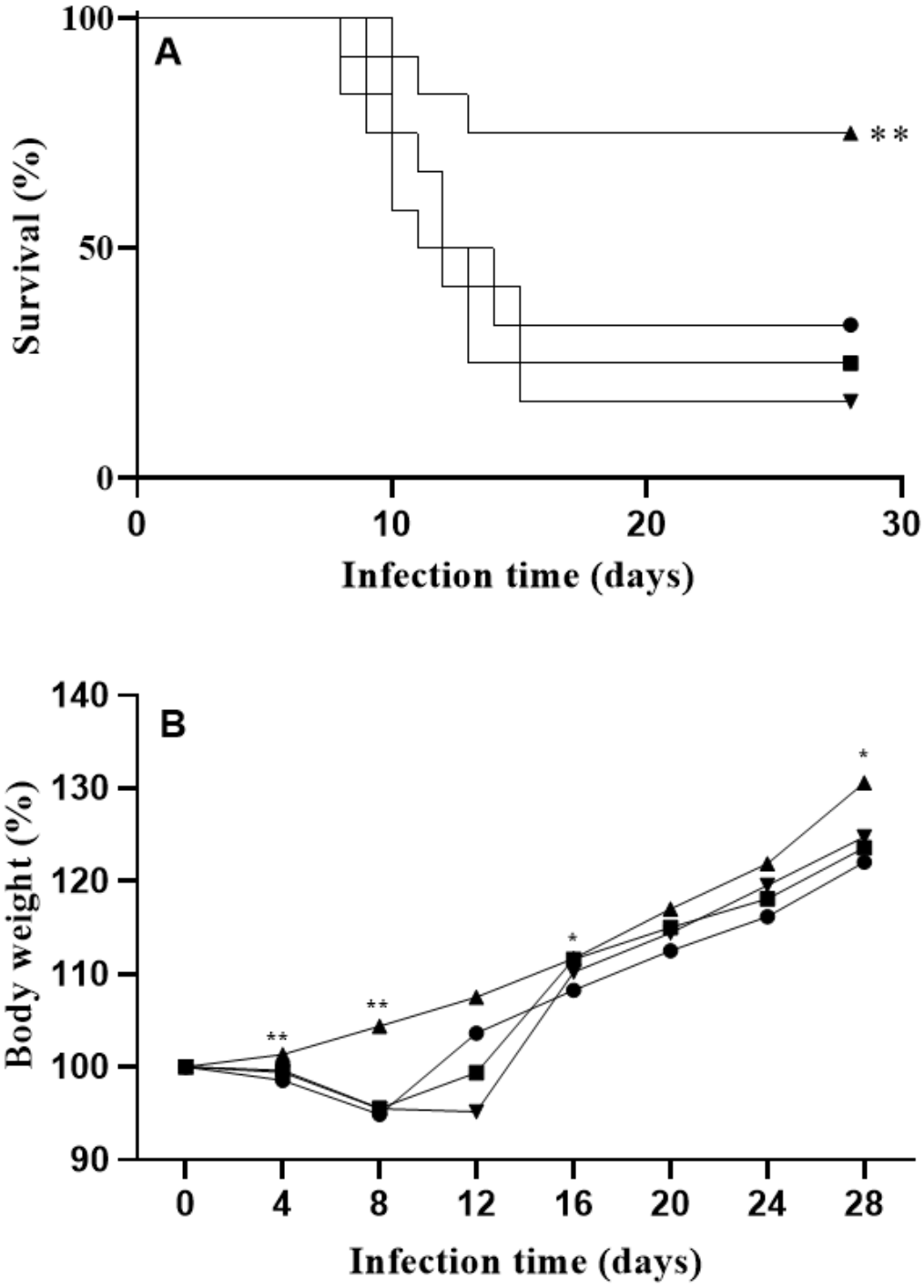

Figure 1

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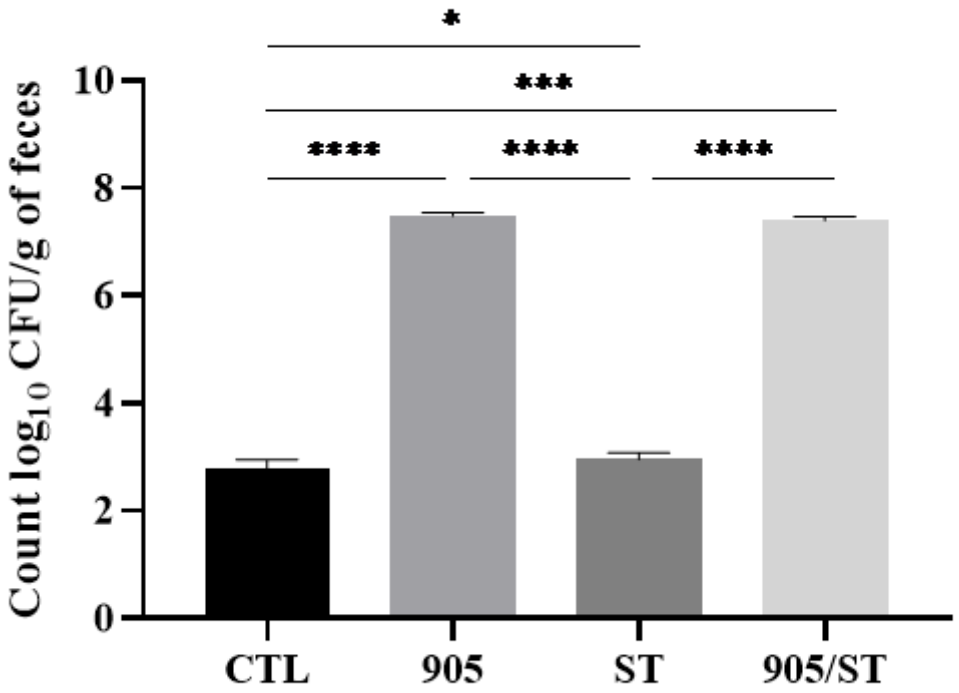

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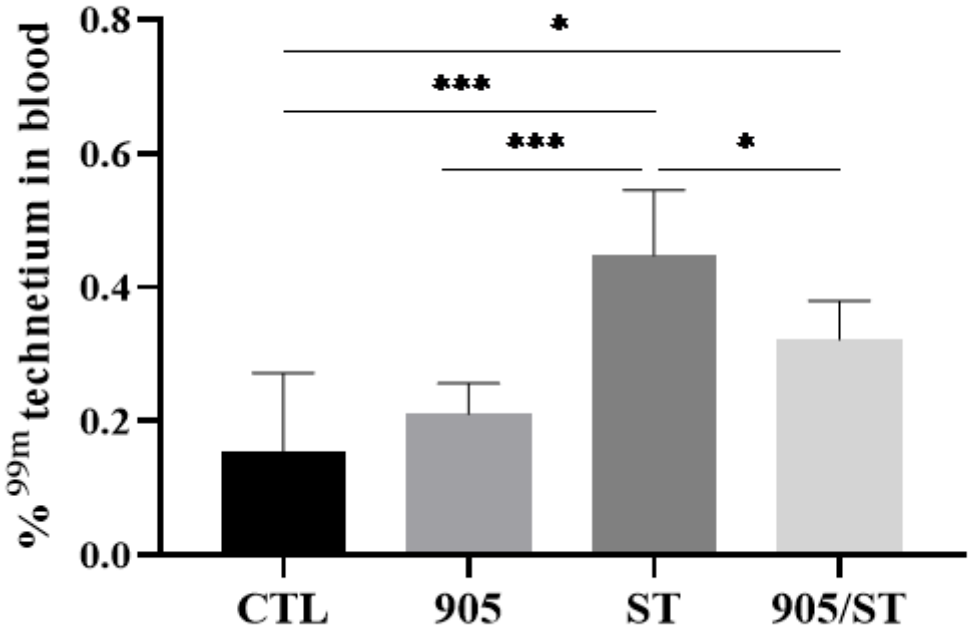

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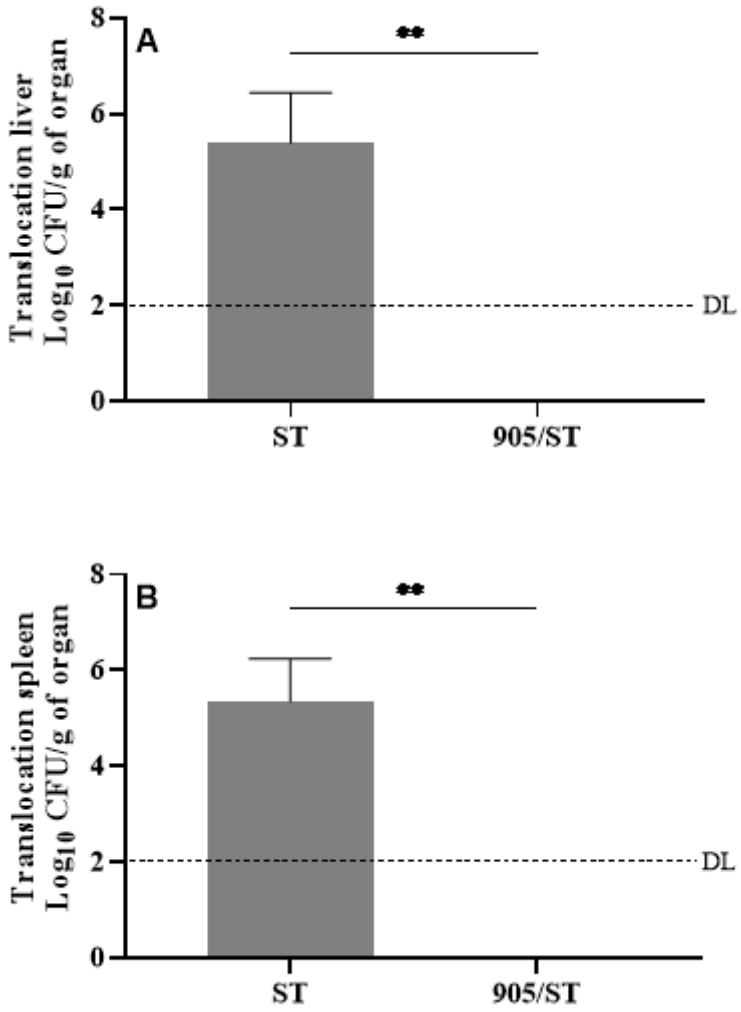

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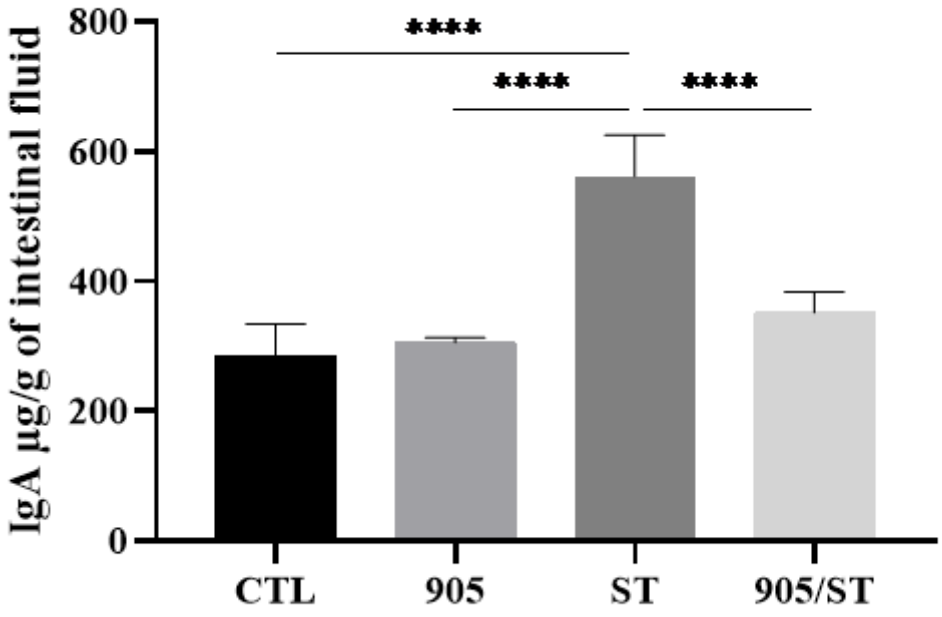

Figure 5

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