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# Gut-borne *Saccharomyces cerevisiae*, a promising candidate for the formulation of feed additives, modulates immune system and gut microbiota

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**RESEARCH ARTICLE**

## Abstract

The aim was to evaluate the effect of *Saccharomyces cerevisiae* RC016 on immune parameters and gut microbiota in healthy mice. Animals received *S. cerevisiae* RC016 for 10 days. Microbial translocation to liver and changes in some bacterial populations in caecum were determined. Immune stimulation was assessed at gut level (measure of immunoglobulin A $(IgA)^+$ cells and luminal cytokine profile) and by evaluating the activity of peritoneal macrophages. Oral administration of *S. cerevisiae* RC016 did not induce microbial translocation to liver. Mice that received yeast increased the number of $IgA^+$ cells in their intestines, the phagocytic activity of peritoneal macrophages and decreased tumour necrosis factor alpha (TNF-α) levels in the small intestine with increases of interleukin-10/TNF-α ratio. Administration of *S. cerevisiae* RC016 caused the decline of a logarithmic unit for *Enterobacteriaceae* counts compared to the control. The immune and gut microbiota modulation observed demonstrates that *S. cerevisiae* RC016 is a promising candidate for the formulation of feed additives to improve animal productivity. The beneficial *in vivo* effects observed for the potential probiotic *S. cerevisiae* RC016 with previously reported mycotoxin-binding properties, demonstrated that this strain could be suitable to be included in a novel product to improve animal productivity, with both probiotic and mycotoxin-binding properties. However, studies in the specific host will be necessary to confirm this potential.

**Keywords:** beneficial microbes, cytokine, dietary supplements, probiotics, immune modulation

## 1. Introduction

The use of antibiotics in food-producing animals has significantly improved animal health by decreasing mortality and diseases. In addition to their therapeutic use, antibiotics are also deployed in animals for prophylaxis and growth promotion. Livestock is closely associated with microorganisms since the gut microbiota of the animals plays an important role in their overall health, productivity and well-being (Callaway *et al.*, 2008; Ley *et al.*, 2008). The legislation of the European Union banned antibiotics as growth promoters in food animals because bacterial pathogens of humans and animals have developed and shared a diversity of antibiotic resistance mechanisms which can be easily spread within microbial populations (Cogliani *et al.*, 2011). In recent years, different alternatives, such as probiotics, have been explored. They represent a potentially significant therapeutic or preventive safe advance in animal production. However, the mechanisms responsible for probiotic effects have not been completely revealed yet. According to the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO, 2001), probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit to the host.

Major mechanisms of action for probiotics consist in epithelial barrier improvement, inhibition of pathogen

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adhesion, competitive exclusion of pathogenic microorganisms, production of antimicrobial substances and host immune system modulation (Bermudez-Brito *et al.*, 2012). The genera most frequently used as probiotics in livestock include *Bacillus* and *Enterococcus* among bacteria and *Saccharomyces* among yeasts (Simon *et al.*, 2001). However, it is important to note that many beneficial microorganisms have not been substantiated as probiotics by experimental evidence yet. In addition, probiotic properties are dependent of each strain and they cannot be extrapolated to other similar microorganisms (same genera and species).

In previous works, the strain *Saccharomyces cerevisiae* RC016 was isolated from the gut of healthy pig. The mycotoxin-binding ability under gastrointestinal conditions and its beneficial properties like co-aggregation and inhibition of pathogenic bacteria as well as the improvement of ruminal fermentation have been reported using *in vitro* assays (Armando *et al.*, 2011, 2012; Dogi *et al.*, 2011). Furthermore, this yeast strain did not induce genotoxicity or cytotoxicity in *in vivo* assays (González Pereyra *et al.*, 2014). To evaluate the potential probiotic properties of this strain, *in vivo* experiments were conducted in mice. The aim of this study was to evaluate the effect of *S. cerevisiae* RC016 on some immune parameters and gut microbiota when orally administered in an experimental model of healthy BALB/c mice. The present work was conducted for the further development of a biological additive with both probiotic and mycotoxin-adsorbent properties to be included in animal feed.

## 2. Materials and methods

### Animals

Male BALB/c mice were obtained from the inbred closed colony maintained at CERELA (Centro de Referencia para Lactobacilos, San Miguel de Tucumán, Argentina). Mice 5 to 6 weeks old, weighing 33±3 g, were housed in groups of five mice per cage under conventional conditions. All animals were maintained in a room with a regular 12-h light/dark cycle at 20±2 °C over the course of the experiment (10 days). Animals received a conventional balanced diet (23% proteins, 6% raw fibre, 10% total minerals, 1.3% Ca, 0.8% P, 12% moisture and vitamins) and water *ad libitum*. The animal protocols were according to the Guide for the Care and Use of Laboratory Animals – National Research Council, 2011. All animal protocols were approved by the Animal Protection Committee of CERELA, and all experiments complied with the current laws of Argentina.

### Experimental groups

Mice were randomly divided into two experimental groups: the control mice received oral phosphate-buffered saline (PBS), while the yeast group mice received an oral suspension of the potentially probiotic *S. cerevisiae* RC016. Each experimental group consisted of five animals and all mice received balanced diet and water *ad libitum.* The experimental protocols were repeated 2 independent times and the samples obtained for the analysis of different data corresponded to 10 mice for each group.

### *Saccharomyces cerevisiae* RC016 administration and experimental design

The yeast strain was previously isolated from pig gut (Armando *et al.*, 2011) and obtained from the Collection Centre at the Universidad Nacional de Rio Cuarto, Argentina. Stock cultures were maintained at -80 °C in 30% (v/v) glycerol. Working cultures were prepared daily from frozen stocks by transfer in yeast extract-peptone-dextrose broth (YPD – 5 g yeast extract, 5 g peptone, 40 g dextrose, 1000 ml water) followed by incubation at 37 °C during 24 h with agitation. After that, cells were harvested by centrifugation at 5,000×*g* for 10 min and washed twice with sterile 0.01 M PBS (pH=7.2).

Daily, each animal was orally administered 0.1 ml of *S. cerevisiae* RC016 suspension in PBS ($1\times10^7$ cfu/ml) using a gavage syringe. The control group received 0.1 ml of sterile PBS. Live body weight was measured at the beginning (day 1) and at the end of the experiment (day 10). The percentage of body weight gain during the experiment was calculated for each mouse. At day 10, mice from each group were euthanized by cervical dislocation. After obtaining peritoneal macrophages, liver and caecum of each mouse were removed aseptically for analysing microbial translocation and intestinal microbiota, respectively. Small intestine and colon and rectum (large intestine) were washed with the appropriate volume to obtain the intestinal contents and washed again before cutting into small pieces for histological procedures.

### Microbial translocation

In order to determine possible sides effects of *S. cerevisiae* RC016 administration, microbial translocation to liver was analysed following the protocols previously described (LeBlanc *et al.*, 2004; Rodriguez *et al.*, 2001). After the assayed period, the livers were aseptically removed, weighed and homogenised in 5 ml of 0.1% peptone solution. Serial dilutions of the homogenate were plated in triplicate in the following agarised media: Mann-Rogosa-Sharp (MRS) for enumeration of *Lactobacilli*, MacConkey for analysis of *Enterobacteriaceae*, and brain-heart infusion (BHI) for enumeration of anaerobic and aerobic microorganisms,

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including possible translocation of the yeast. All these culture media were obtained from Britania (Buenos Aires, Argentina). Bacterial growth was evaluated after incubation of the plates at 37 °C for 24 to 72 h in aerobic and anaerobic (using anaerobic jars and anaerobic gas generating sachets, AnaeroPack® Mitsubishi Gas Chemical Co Inc., Tokyo, Japan) conditions.

### *Ex vivo* phagocytosis assay of peritoneal macrophages

Peritoneal macrophages were obtained according to Valdez *et al.* (2001). Macrophages were aseptically extracted from peritoneal cavity with 5 ml of sterile PBS pH 7.4 containing 100 µg/ml of gentamicin (Gm). Macrophages were washed by centrifugation and resuspended in RPMI 1640 medium (Sigma, St Louis, MO, USA). Phagocytosis assay was performed using a commercial yeast as antigen (*Saccharomyces boulardii* Hansen CBS 5926 from Floratil, MERCK Química, Buenos Aires, Argentina) at a concentration of $10^7$ cells/ml. Equal volumes of opsonised commercial yeast (incubated previously with normal mouse serum) were mixed with $10^6$ cells/ml of macrophage suspensions. The mixture was incubated for 30 min at 37 °C. The assay was performed in duplicate. Phagocytosis was expressed as the percentage of phagocytic macrophages in 200 cells counted using an optical microscope.

### Histological samples

After obtaining the intestinal contents, small and large intestine (colon and rectum) were again washed, cut into small pieces (unopened) and fixed in 10% formaldehyde solution in PBS, pH 7. From the small intestine, three portions (2 cm length each) were selected; one near the stomach, the other from the middle and the last one at the end (before the cecum). From the large intestine, colon and rectum were removed and cut into pieces of approximately 2 cm of length. After fixation (48 h), the tissues were dehydrated and embedded in paraffin following standard methodology (Sainte-Marie, 1962). Serial sections (4 µm) were cut from all the samples using a rotation microtome.

### Determination of immunogloblin $A^+$ cells in the small and large intestine tissues

The number of immunoglobulin A $(IgA)^+$ cells was determined on histological slices from small and large (colon and rectum) intestine using a direct immunofluorescence assay. After paraffin removal using xylene substitute (Alclara, Alwik, Buenos Aires, Argentina) and rehydration in a decreasing gradient of ethanol, paraffin sections (4 µm) were incubated with a 1:100 dilution in PBS of anti-mouse IgA (α-chain) monospecific antibody (developed in goat) conjugated with fluorescein isothiocyanate (FITC), Sigma, St Louis, MO, USA) for 30 min at 37 °C. After incubation, the tissues were washed twice with PBS and mounted with mounting medium (glycerol:PBS, 9:1). The samples were observed with an epifluorescence microscope (Axiostar Plus FL, Carl Zeiss GmbH, Oberkochen, Germany). The assay was performed in duplicate and the control slides were incubated without any antibody (PBS) or with normal goat serum (diluted 1:100 in PBS) to confirm the absence of non-specific fluorescence. Fluorescent cells in the mucosa of small and large intestine (colon and rectum) samples were counted by blind counts. Thirty fields of view at 1000× magnification were evaluated in the small as well as in the large intestine samples by covering at least 10 random fields in each portion of the intestine. Areas with big organised lymphoid aggregates were excluded. Results were expressed as the number of positive fluorescent cells per ten fields of vision.

### Determination of cytokines in the intestinal fluid

Intestinal contents were collected from the small and large (colon and rectum) intestines of mice with 500 µl of cold PBS, maintained on ice and then centrifuged at 5,000×*g* for 15 min at 4 °C. The supernatants were recovered and stored at -80 °C until cytokine determinations. These samples were analysed with the Cytometric Bead Array Mouse Inflammation Kit (CBA, BD Bioscience, San Diego, CA, USA) to measure interleukin (IL)-6, IL-10, monocyte chemoattractant protein-1 (MCP-1), interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-a), and IL-12p70 levels in a single sample, following the manufacturer's instructions. Cytokine quantification was performed using a FACS Calibur flow cytometer (BD Bioscience), and data acquired for each sample and standard (provided by the manufacture) were analysed using the FCAP Array V1.0.1 software (BD Bioscience). The assay was performed in duplicate. The results were expressed as concentration of each cytokine in the intestinal fluid (pg/ml).

### Analysis of the intestinal microbiota

The caecum from mice in control and yeast groups were aseptically removed, weighed and placed in sterile tubes containing 5 ml of 0.1% peptone solution. The samples were immediately homogenised under sterile conditions. Serial dilutions of the homogenised samples were performed and aliquots (0.1 ml) of dilutions were plated in duplicate in the following agarised media obtained from Britania (Buenos Aires, Argentina): Reinforced Clostridial Agar (RCA) for total anaerobic bacteria, MRS for total *Lactobacilli*, and MacConkey for total *Enterobacteriaceae*. MacConkey and MRS agar plates were aerobically incubated at 37 °C for 24 and 48 h, respectively. RCA plates were anaerobically incubated at 37 °C for 72-96 h using anaerobic jars and anaerobic gas generating sachets (AnaeroPack® Mitsubishi Gas Chemical Co Inc.). After incubation, the number of cfu was manually counted on the plate with the appropriate dilution of the sample (plates with 30-300 colonies).

### Statistical analysis

Results were expressed as the mean values of independent results ± standard deviation. Ten mice of each group were sacrificed and samples were collected (n=10). The results were analysed by test of mean differences. The analysis was conducted using PROC GLM in SAS (SAS Institute, Cary, NC, USA).

## 3. Results

### Evaluation of side effects associated with *Saccharomyces cerevisiae* administration

No significant differences were observed in the gain of body weights of mice receiving the *S. cerevisiae* RC016 compared to the control group at 10 days of experiment (Table 1). The results obtained from microbial translocation assay showed that 10 days of *S. cerevisiae* RC016 oral administration did not correlate with either the passage of gut microorganisms or the assayed yeast from the intestinal lumen to distant organs such as liver. Microbial growth was not observed in the samples obtained from the liver of control mice or mice given *S. cerevisiae* RC016 (data not shown).

### Effect of *Saccharomyces cerevisiae* RC016 administration on peritoneal macrophages phagocytic activity

The results obtained from phagocytic activity of peritoneal macrophage are shown in Figure 1. The administration of *S. cerevisiae* RC016 to healthy mice during 10 days significantly increased ($P<0.001$) the peritoneal macrophages phagocytic activity (33±6%) compared to the control group (17±2%).

### Determination of immunoglobulin A$^+$ cells in the small and large intestine tissues

When analysing the effect of *S. cerevisiae* RC016 administration on the intestinal IgA producing cells, it was observed that this yeast strain induced significant increases in the number of IgA$^+$ cells ($P<0.05$) in both the small and large (colon and rectum) intestine of mice after 10 days of administration, compared to the control group (Figure 2).

**Table 1. Changes in body weight in mice that received a suspension of *Saccharomyces cerevisiae* RC016 (RC016).[1]**

| | Control (g) | RC016 (g) |
|---|---|---|
| Day 1 | 33.08±2.81 | 33.54±3.72 |
| Day 10 | 36.21±3.07 | 35.35±4.35 |
| Change (%) | 8.64±1.79 [a] | 6.73±1.99 [a] |

[1] Results are mean ± standard deviation (n=10). Control mice received a phosphate buffered saline solution. The same letter for the obtained values indicates no significant differences ($P<0.05$) between the groups.

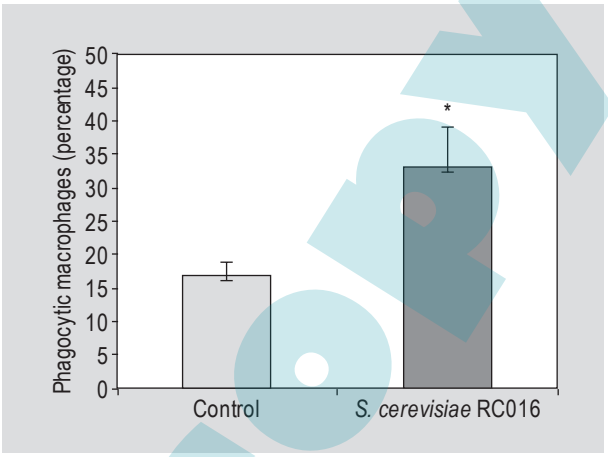


**Figure 1. *Ex vivo* phagocytic activity of peritoneal macrophages from mice that received daily a suspension of *Saccharomyces cerevisiae* RC016 or phosphate buffered saline (Control) during 10 days. The values are expressed as the percentage of phagocytic macrophages in 200 cells counted using an optical microscope. Each bar represents the mean ± standard deviation (n=10). * Statistically different compared to the control ($P<0.001$).**

### Determination of cytokines in the intestinal fluids

Results for determination of IL-10, MCP-1, IL-12p70 and TNF-α in the small intestine contents are shown in Figure 3. A decrease in TNF-α levels was observed in the samples obtained from small intestine of mice that received *S. cerevisiae* RC016 compared to the control group. In addition, the analysis of IL-10/TNF-α ratio in these samples showed that yeast administration resulted in an increase of the anti-inflammatory /pro-inflammatory cytokine ratio ($P<0.05$) compared to the control group (Figure 3E) However, no significant differences were observed for IL-10/IL-12p70 ratio between the groups (Figure 3F). Results for cytokine determinations in the large intestine (colon and rectum) are shown in Figure 4. No significant modifications were observed for IL-12p70 and MCP-1 when compared test and control group (Figures 4A and 4B). IL-10 concentrations showed a high variability between the mice (Figure 4C) which was reflected in the IL-10/IL-12p70 ratio (Figure 4D), without significant differences between the groups. TNF-α levels were under the sensitivity of the assay for these samples and IFN γ and IL-6 for both small and large intestine samples.

### Analysis of the intestinal microbiota

The results obtained demonstrated that administration of *S. cerevisiae* RC016 to healthy mice during 10 days resulted in a decrease of one logarithmic unit for *Enterobacteriaceae*

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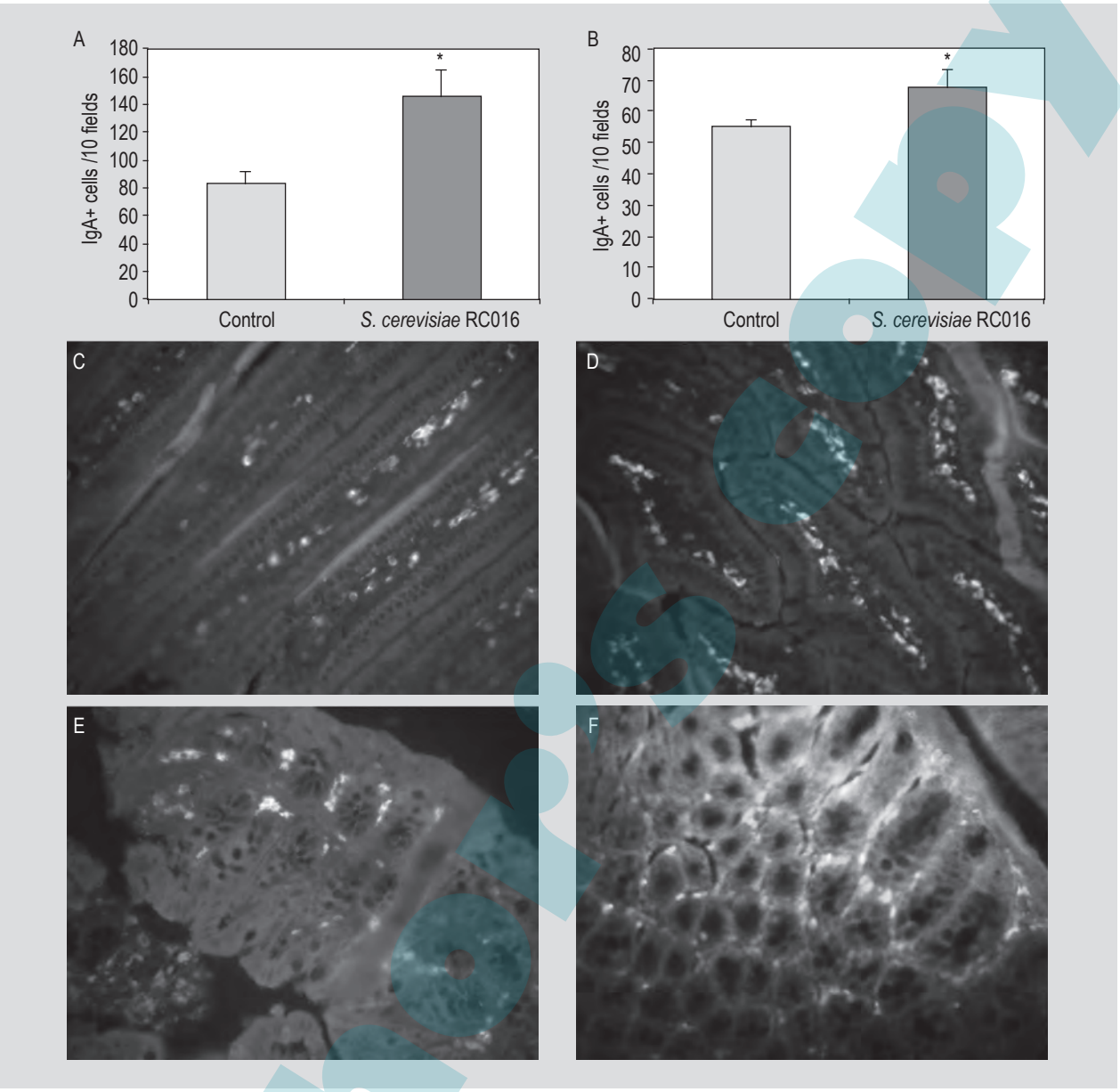


**Figure 2. Direct immunofluorescence assay for IgA$^+$ cells. IgA$^+$ cells were determined by direct immunofluorescence in slides from the small intestine (A) and large intestine (colon and rectum) (B) of mice that received a suspension of *Saccharomyces cerevisiae* RC016 or phosphate buffered saline (Control) during 10 days. Results are expressed as number of positive cells counted in 10 fields of vision at 1000× magnification. Each bar represents the mean ± standard deviation (n=10). * Mean values differ significantly with the control ($P$<0.05). Representative microphotographs (400×) from a mouse of each group are showed: (C) Small intestine of control mouse; (D) Small intestine of mouse given *S. cerevisiae* RC016; (E) Large intestine of control mouse; (F) Large intestine of mouse given *S. cerevisiae* RC016.**

cfu (5.53±0.31) compared to the control group (4.85±0.14) (Figure 5). No significant changes were observed for the other bacteria studied comparing the two groups ($P$<0.05).

## 4. Discussion

The ban by the legislation of the European Union for the use of antibiotics in food animals has put increasing pressure on animal producers to adopt alternative strategies to reduce or eliminate antibiotics in feeds. Among the proposed alternatives, probiotics are considered good candidates because they have the potential to improve the gut barrier properties (Madsen *et al.*, 2001) and to stimulate systemic and intestinal immunity to defend the host against infections (Dugas *et al.*, 1999).

*S. cerevisiae* has the GRAS (Generally Recognised As Safe) status from the US Food and Drug Administration.

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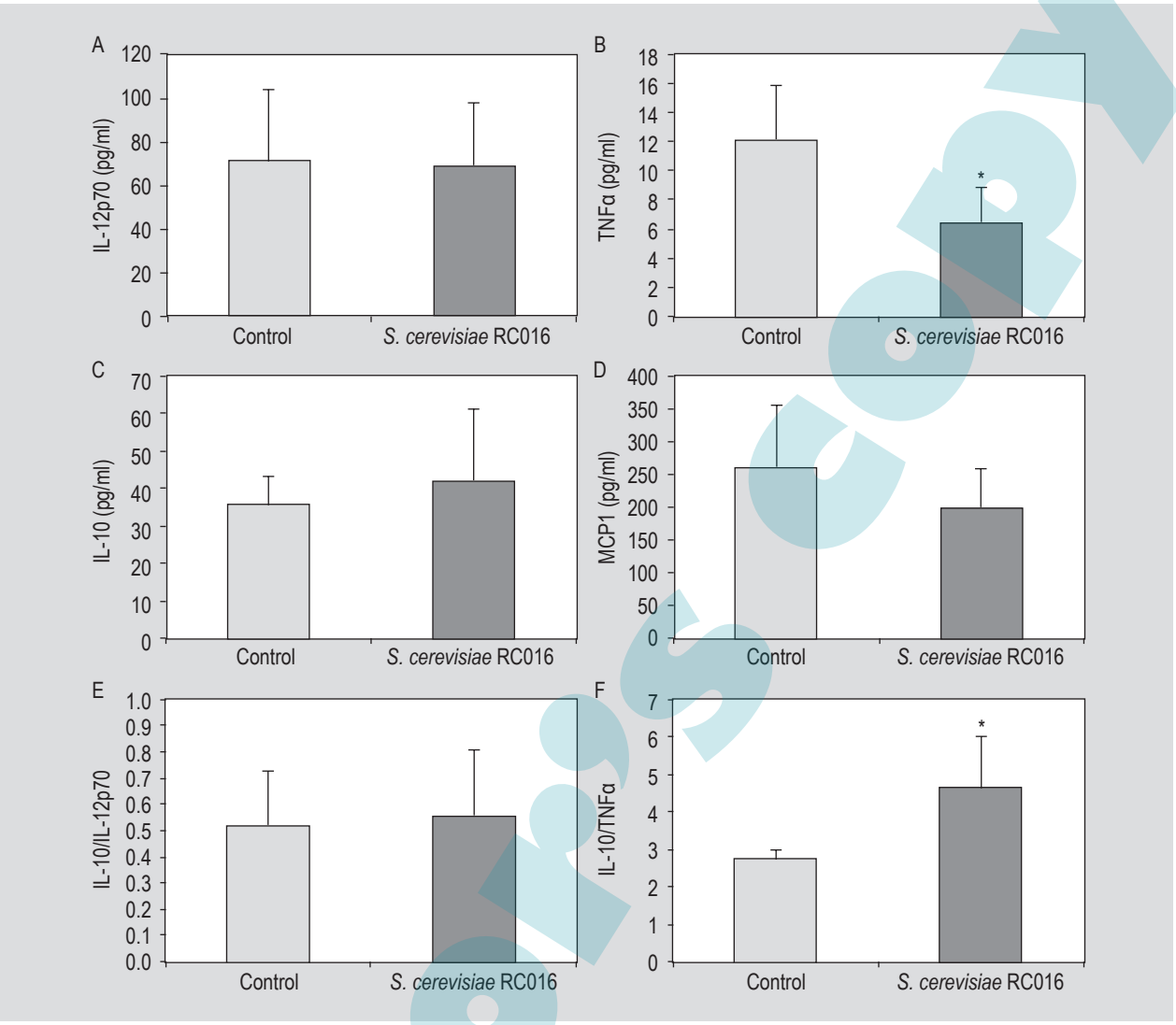


**Figure 3. Determinations of cytokines in small intestine contents of mice that received a suspension of *Saccharomyces cerevisiae* RC016 or phosphate buffered saline (Control) during 10 days. Samples were analysed for IL-12p70 (A), TNF-α (B), IL-10 (C) and MCP-1 (D) using a cytometric bead array kit. Results are expressed as concentration of each cytokine (pg/ml) in the intestinal fluid. The ratio between IL-10 and the pro-inflammatory cytokines TNFα (E) or IL-12p70 (F) were also analysed. Each bar represents the mean ± standard deviation (n=10). * Mean values significantly differ from the control (*P*<0.05).**

However, in the selection of a probiotic microorganism the safety of each strain needs to be evaluated. In the present work it was demonstrated that *S. cerevisiae* RC016 administration to healthy mice did not induce negative side effects such as intestinal microbiota translocation to the liver or loss of body weight. With respect to body weight, growth promotion is a desired quality for probiotics in livestock feed and poultry, as was reported in broiler chickens supplemented with a yeast probiotic preparation (Aluwong *et al.*, 2013). In our study, the beneficial effects of *S. cerevisiae* RC016 administration to mice were not associated to body weight increase, similar to the results reported recently for yeast supplementation on the growth performance of lambs (Soren *et al.*, 2013). However, with our study, we cannot discard that *S. cerevisiae* RC016 can affect body weight in young animals, such as weaned piglets or even under longer periods of administration. Body weight was considered as a safety parameter to discard negative effects, such as diarrhoea, that can be associated with microbial supplementation and causes loss of body weight. Similarly, microbial translocation to liver was evaluated to investigate if the potential probiotic yeast causes an intestinal microbial imbalance and / or alterations of intestinal barrier associated with the passage of gut microorganisms to other organs such as liver. This study also showed that *S. cerevisiae* RC016 was not found in the liver of the mice that received this yeast. In addition, previous studies have reported absence of genotoxicity and cytotoxicity for this yeast strain in a rat experimental model (Gonzalez Pereyra *et al.*, 2014).

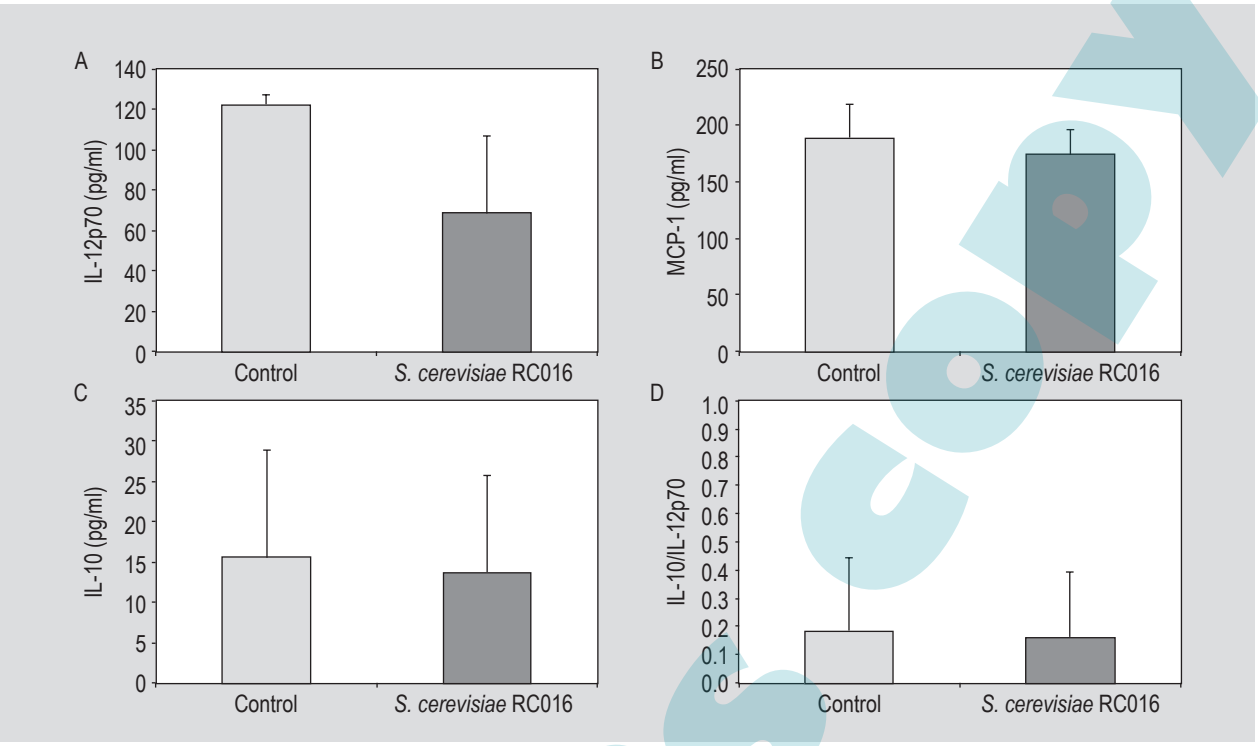


**Figure 4. Determinations of cytokines in large intestine contents of mice that received a suspension of *Saccharomyces cerevisiae* RC016 or phosphate buffered saline (Control) during 10 days. Samples were analysed for IL-12p70 (A), MCP-1 (B) and IL-10 (C) using a cytometric bead array kit. The results are expressed as concentration of each cytokine (pg/ml) in the intestinal fluid. The ratio between IL-10 and the pro-inflammatory cytokine IL-12p70 (D) was also analysed. Each bar represents the mean ± standard deviation (n=10).**

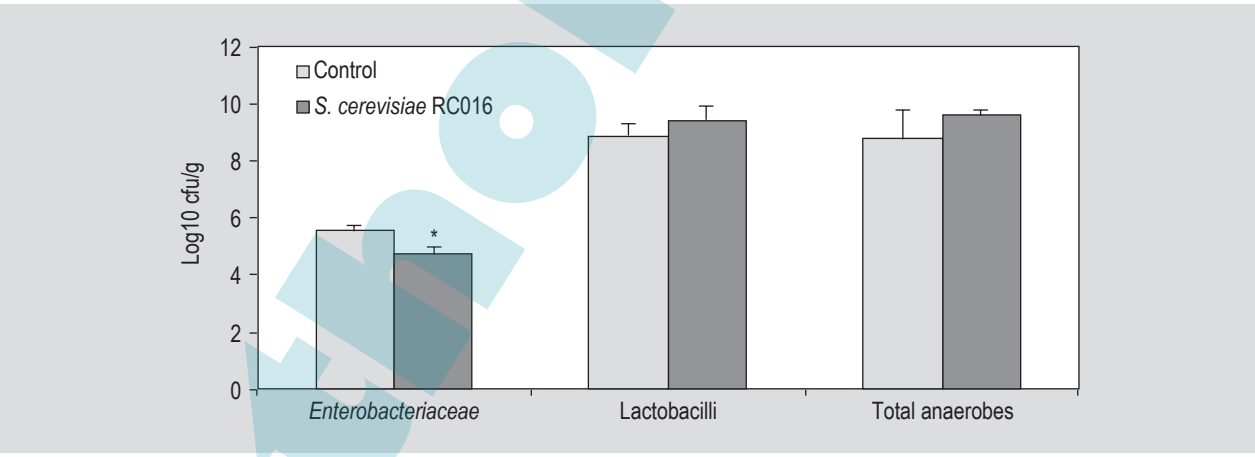


**Figure 5. *Enterobacteriaceae*, lactobacilli and total anaerobes in samples from caecum of mice that received a suspension of *Saccharomyces cerevisiae* RC016 or phosphate buffered saline (Control) during 10 days. Determination was made by conventional culture techniques. Colony counts are expressed as $\log_{10}$ cfu/g caecum. Each bar represents the mean ± standard deviation (n=10). * Statistically different compared to the control ($P$<0.05).**

The health-promoting properties attributed to probiotics are multiple and include their capacity to activate/modulate the host immune system (De Moreno de LeBlanc *et al.*, 2008; Dogi *et al.*, 2008; Galdeano *et al.*, 2007; Perdigón *et al.*, 2000). In the present work it was demonstrated that the oral administration of *S. cerevisiae* RC016 was able to stimulate the mice´s immune system, not only in the intestine but also by increasing the phagocytic activity of peritoneal macrophages. Similar results were observed in mice that received commercial probiotic fermented milk which had increased phagocytic activity of peritoneal macrophages until day 14$^{th}$ of administration (De Moreno

de Leblanc *et al.*, 2008). The increased phagocytic activity of peritoneal macrophages can also be associated with the potential protection against pathogens, as was reported for the probiotic *Lactobacillus casei* CRL 431 in a mouse model of *Salmonella enterica* serovar Typhimurium infection (De Moreno de LeBlanc *et al.*, 2010).

At the intestinal level, *S. cerevisiae* RC016 administration increased the number of $IgA^+$ cells. This was observed in both, small and large (colon and rectum) intestine. The main mechanism of protection given by the GALT is humoral immune response mediated by secretory IgA (s-IgA), which prevents the entry of potentially harmful antigens and also interacts with mucosal pathogens without potentiating damages. In contrast to this humoral response, innate immune cells, including neutrophils and macrophages can also act against pathogens and induce protective but destructive responses (Lebeis *et al.,* 2008). An increasing number of probiotic strains were shown to increase s-IgA (Delcenserie, *et al.*, 2008; Thomas and Versalovick, 2010), therefore the stimulation of IgA producing cells is often considered a desirable property in the screening of probiotic microorganisms. Beneficial effect of probiotics is also associated with down regulation of excessive inflammatory response; therefore the analysis of some pro-inflammatory and regulatory cytokines was performed in intestinal fluids obtained from small and large intestine of mice. It is important to note that *S. cerevisiae* RC016 administration to healthy mice did not induce increases of the pro-inflammatory cytokines, such as TNF-α and IL-12p70. The overproduction of these cytokines may cause pathological inflammatory response and it is also desirable that probiotic administration can modulate the production of these cytokines in pathological conditions. In this sense, TNF-α is an important pro-inflammatory molecule with pleiotropic functions in intestinal inflammation. In the present work, a significant decrease of TNF-α was observed after yeast administration. This is an important result since immunological responses that occur during the weaning process in pig involve up-regulation of proinflammatory cytokines, mainly TNF-α (Pié *et al.*, 2004). Pro-inflammatory cytokines have an influence on intestinal integrity and epithelial function which are related to permeability and nutrient transport (McKay and Baird, 1999).

The chemokine MCP-1 was analysed because it is responsible for the recruitment of monocytes, basophils, natural killer cells, T lymphocytes and also dendritic cells and memory T lymphocytes (Rydstrom and Wick, 2009). An increase of MCP-1 can be associated with pro-inflammatory response (Reinecker *et al.*, 1995). The analysis of this chemokine showed that even while *S. cerevisiae* RC016 administration increased the phagocytic activity of peritoneal macrophages, it did not increase the recruitment of these cells by MCP-1 at the intestinal level. However, the recruitment via other ways is still possible and should be evaluated in future studies. IL-10 is a pluripotent cytokine and the most important anti-inflammatory cytokine found within the mammalian immune response. All the activities of IL-10 lead to the inhibition of the production of pro-inflammatory mediators while enhancing the production of anti-inflammatory mediators (De Moreno de LeBlanc *et al.*, 2011). While IL-10 concentration did not increase significantly in the intestinal fluids of mice given *S. cerevisiae* RC016, compared to the control, an increased IL-10/TNFα ratio was observed in small intestine fluid from the mice receiving yeast. Although these results were obtained in healthy mice, they allow suggesting the use of *S. cerevisiae* RC016 as a probiotic strain that could successfully modify the mucosal immune response by modulating gut inflammation. Nevertheless, this should be corroborated using models of intestinal inflammation.

Yeast has been used for decades and its use has resulted in improved animal production, health and increased growth. *S. cerevisiae* has been administered as preventive or therapeutic agent for diarrhoea and other gastro-intestinal disturbances in both, ruminant and nonruminant species (Chaucheyras-Durand *et al.*, 2008; Czerucka *et al.*, 2007). Previous studies have demonstrated that *S. cerevisiae* RC016 has been able to co-aggregate and inhibit enteric pathogenic bacteria *in vitro* (Armando *et al.*, 2011) and to modulate ruminal microbiota (Dogi *et al.*, 2011). In the present work, *S. cerevisiae* RC016 administration was associated with the diminution of one logarithmic unit for *Enterobacteriaceae* in the mouse model. This result can be due to the presence of live yeast in the intestinal environment or to some components of the yeast that can act by favouring or decreasing the growth of certain microorganisms. Future studies should be done to figure out the mechanisms implicated. This ability (even if it was a small decrease of certain bacteria in the model) to modulate intestinal microbiota in healthy animals, reducing potential pathogens is a desirable property for a probiotic strain used in animal production and it should be evaluated in the host for which it is proposed. Weaned piglets are highly vulnerable to pathogenic enteric conditions such as post-weaning diarrhoea caused by serotypes of enterotoxigenic *Escherichia coli* (Pluske, 2013; Rist *et al.*, 2013).

## 5. Conclusions

The present study describes the results of an *in vivo* trial for a potential probiotic *S. cerevisiae* strain isolated from the pig intestine, which previously demonstrated beneficial and mycotoxin-binding properties *in vitro*. The beneficial *in vivo* effects observed on the host immune response after the intake of *S. cerevisiae* RC016 are promising since this strain could be suitable for the formulation of feed additives to improve animal productivity with both, probiotic and mycotoxin-binding properties. The use of

microbial additives with beneficial properties to the host, instead of chemical products, is a safer and eco-friendly option to increase animal productivity with a minimised environmental impact.

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