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Saccharomyces boulardii and *Candida albicans* experimental colonization of the murine gut

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Saccharomyces boulardii has been and continues to be extensively used as a probiotic, with only rare associations with fungemia. This study evaluated the virulence of this yeast when given as a probiotic, and its role in preventing gastrointestinal (GI) colonization by *Candida*. Adult male Crl:CD1 (ICR) BR mice were given *S. boulardii* orally in three different doses or normal saline for 14 days. Stool cultures were performed at the time of discontinuation of yeast administration, as well as 1 and 2 weeks later. Gut colonization was proportional to the given dose but lasted only 1 week and no dissemination of the yeast was detected. *S. boulardii* was also given for 2 and 4 weeks to mice fed chow containing *Candida albicans*. *S. boulardii* in the gut did not affect *Candida* GI colonization. These findings suggest that oral administration of *S. boulardii* induces a substantial but short term increase of this yeast in the intestinal lumen and administration of the probiotic does not prevent subsequent GI colonization by *C. albicans*.

Keywords probiotics, *Saccharomyces boulardii*, gastrointestinal tract, *Candida albicans*, mice

Introduction

Saccharomyces boulardii, a non-pathogenic yeast when used as a probiotic, has been shown to be effective in the prevention and/or treatment of intestinal disorders, including antimicrobial-associated diarrhea, recurrent *Clostridium difficile* disease, acute diarrhea in adults and children induced by a variety of enteric pathogens, traveler's diarrhea and relapses of Crohn's disease or ulcerative colitis [1–4].

Several studies have shown that oral administration of *S. boulardii* is a safe and well-tolerated treatment [1,5]. However, there are also several documented reports of *S. boulardii* fungemia and septicemia associated with oral administration of this organism when used as a probiotic in immunocompetent and immunocompromised patients [6–10].

Experiments in animals have shown that *S. boulardii* remains in the gut only during the period of its oral administration [11,12]. If given in high doses, *S. boulardii* can translocate from the gut lumen across the intestinal mucosa to mesenteric lymph nodes, liver and spleen [13,14].

The aim of the present study was to assess the virulence and the potential dissemination of the extensively used, commercially available strain of *S. boulardii*, when given orally in different doses in an experimental mouse model. In addition, we wanted to investigate the effects of *S. boulardii* on the gastrointestinal (GI) colonization by *C. albicans* in an established experimental model which successfully mimics the function of the human gut [15].

Materials and methods

Five groups, each containing 40 3-month-old, male Crl:CD1 (ICR) BR mice, weighing approximately 30 g each, were used in each experiment. All experiments were performed in triplicate (in total 600 animals were used).

Saccharomyces boulardii commercial formulation was given to the animals (Ultra-levure, Biocodex, France). The dose schedules were equivalent to those of humans and were calculated by the method of Freireich *et al*. [16], as shown in Table 1. Thirty mice in each of the first three groups (A, B, C) were given 300 μl suspension per os

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containing one of the 3 different dosage schedules of the probiotic for 14 days, as shown in Table 1. The remaining 10 mice of each group served as controls and received normal saline at the same schedule, volume (300 μl), and route.

Baseline stool cultures for yeasts were performed for all animals of the groups A, B, and C. Quantitative stool cultures were performed on the last day of treatment, and 1 and 2 weeks after the end of treatment.

On the last day of treatment, 10 mice from each group were randomly selected (by a technician not participating in the study), sacrificed by cervical dislocation and dissected. The lungs, heart, kidneys, liver and spleen were separately weighed and homogenized in 10 ml of saline solution. One hundred microlitres from the resulting suspension of each organ were inoculated onto plates containing Columbia agar with 5% sheep blood (BioMerieux, Marcy-l'Etoile, France) and Sabouraud dextrose agar with chloramphenicol (Diagnostics Pasteur, Marnes le Coquette, France) and incubated at 37°C for 48 h.

Histopathological examination was performed on all organs by the same pathologist. The biopsy specimens were fixed rapidly in 10% formalin and were subsequently embedded in paraffin and stained with periodic acid-Schiff (PAS) and Gomori's methenamine silver (GMS) to detect tissue invasion by the yeasts.

The 40 mice in the fourth group were given per os a 300 μl suspension containing 1.28 mg of the probiotic every 12 h for 2 weeks. Subsequently, starting on the day of discontinuation of the administration of the probiotic, the mice were fed special chow containing *C. albicans* at a median concentration of 1.5×10^7 CFU/g for a period of 2 weeks, as previously described [15]. Special attention was paid by animal caretakers that mice were consuming the appropriate quantity of food which was provided on a daily basis after consumption of the food from the previous day.

Baseline stool cultures for yeasts were performed for all animals of the fourth group. Quantitative stool cultures were performed on the last day of the probiotic administration, and 1, 2, 3, and 4 weeks after its discontinuation.

The 40 mice in the fifth group were given per os a 300 μl suspension of 1.28 mg of the probiotic every 12 h for 4 weeks. On day 15 of treatment the mice were given chow containing *C. albicans* at a median concentration of 1.5×10^7 CFU/g for a period of 2 weeks [15].

Baseline stool cultures for yeasts were performed for all animals of the fifth group. Quantitative stool cultures were performed 2, 3, 4, 5 and 6 weeks after the initiation of the probiotic treatment.

Table 1 Equivalence of probiotic dosage schedules in humans and mice.

Daily dosage schedule	
70 kg human	30 g mice
250 mg q[a] 12 h	1.28 mg q 12 h
500 mg q 12 h	2.57 mg q 12 h
1000 mg q 12 h	5.14 mg q 12 h

[a]q, every.

Stool cultures

An average of five pieces of stool, weigh approximately 0.1 gram, were collected each time from each animal. Four grams of feces were collected from each group of 40 mice and cultured by mixing each gram of stool with 9 ml of sterile isotonic saline (0.9%) and emulsifying in a vortex mixer. Samples were then serially diluted in normal sterile saline. Susequently, 0.1 ml from each dilution was inoculated onto the selective, differential medium, Colorex Candida agar (E&O Laboratories Limited, Scotland) and incubated at 37°C for 48 h. Quantitation and identification of different yeast colonies were performed at the end of this incubation period with the lowest detectable concentration of yeasts measuring 10^2 CFU/g of stool.

Approval for all experiments was granted from the relevant ethics committee.

Statistical analysis

Statistical analysis was performed with the use of graph Pad Prism software (version 5). We analyzed the median and range of yeast CFU/gr of stool for each group of mice. Statistically significant differences in yeast CFUs across different groups of mice were analyzed by using the Mann-Whitney for two-sample comparisons and the Kruskal-Wallis test for several independent samples. P values of <0.05 were considered statistically significant for all comparisons.

Results

While yeasts were not recovered in baseline cultures, samples from the first three groups (A, B, C), taken on day 14

Table 2 Concentration of *Saccharomyces boulardii* determined by stool cultures.

	Median *S. boulardii* concentrations (range) ($\log_{10}$ CFU/g of stool)		
Time period	Group A	Group B	Group C
Day 14 of administration	5.2 (4.9–5.5)	6.8 (6.7–6.9)	7.7 (7.0–8.0)
1 week after probiotic d/c[a]	2.1 (0.0–2.2)	2.2 (2.0–2.3)	2.4 (2.0–2.6)
2 weeks after probiotic d/c	0[b]	0	0

[a]d/c: discontinuation.

[b]Baseline values were 0 in all cases. Values of 0 indicate that cultures did not reveal *S. boulardii* in the stools. Cultures from control mice resulted in values of 0 at all phases.

of treatment with *S. boulardii* did yield the yeasts at concentrations which varied depending on the dose administered. The mice receiving the high dosage (group C) showed a statistically significant increase in the GI concentration of *S. boulardii* of 7.7 $\log_{10}$ CFU/g of stool when compared to the mice administered low doses (group A) and median concentrations (group B) dosages ($P = 0.005$ for group C vs. group A $P = 0.022$ for group C vs. group B). The median dosage caused an increase of 6.8 $\log_{10}$ CFU/g of stool and the low dosage caused a minor increase of 5.2 $\log_{10}$ CFU/g of stools. Stool cultures taken 1 week after the end of treatment showed a significant reduction of the yeast concentrations in all groups (Table 2). No *S. boulardii* was recovered from stool specimens taken 2 weeks after discontinuation of treatment in all groups (Table 2). No *S. boulardii* was detected in the stools of mice given saline, serving as controls. There was no histopathological or microbiological evidence of *Saccharomyces* dissemination and/or infection in any of the examined organs or in cultures initiated with portions of tissue from the same organs.

Regarding the fourth group, no yeasts were isolated in baseline cultures. Stool specimens taken on day 14 of treatment with *S. boulardii* showed that the level of GI colonization of mice caused an increase of 5.1 $\log_{10}$ CFU/g of stools. Stool cultures taken 1 week after the end of treatment and the initiation of the special diet containing *C. albicans* showed a significant increase in the concentration of *C. albicans* to the level of 7.8 $\log_{10}$ CFU/g of stools and reduction of *S. boulardii* to 2.0 $\log_{10}$ CFU/g of stools. No *S. boulardii* was isolated from the stool samples taken 2, 3, and 4 weeks after discontinuation of treatment. In contrast, the concentration of *C. albicans* gradually declined significantly, reaching a concentration of 4.25 $\log_{10}$ CFU/g of stools ($P < 0.0001$; Table 3).

Regarding the fifth group, again no yeast was found in baseline cultures. Stool cultures done 2 weeks after the probiotic initiation showed that faecal *S. boulardii* concentration was 5.1 $\log_{10}$ CFU/g of stool, while 2 weeks later it had increased to 7.2 $\log_{10}$ CFU/g of stools, but disappeared a week after the discontinuation of using it as a probiotic. On day 14 of special diet, the concentration of *C. albicans* increased to 5.5 CFU/g of stools. Stool cultures done 14 days after the end of the *C. albicans* supplemented diet showed that *C. albicans* persisted in a concentration of 4.2 $\log_{10}$ CFU/g of stools (Table 4).

Discussion

S. boulardii, the probiotic strain of *S. cerevisiae*, is considered to be non-pathogenic and is widely used for the prevention and/or treatment of several types of diarrhea [1–3,17]. The benefits of the probiotic are thought to be related to direct enzymatic effects, modulation of the gut endogenous flora and enhancement of the immune response [18,19].

Oral administration of *S. boulardii* in the form of capsules containing the yeast in a lyophilized form has traditionally been considered safe. However, several reports of *S. cerevisiae* fungemia associated with its probiotic use have been published [6–10]. Lherm *et al*. [6] reported seven cases of systemic fungal infection in a series of 1395 intensive care unit (ICU) patients treated with *S. boulardii*. The authors concluded that the incidence of fungemia might be approximately five per 1000.

Most patients with *S. boulardii* fungemia were immunosuppressed due to corticosteroids or immunosuppressive agents, hematopoietic stem cell transplantation, malignant haematological diseases, neutropenia, diabetes, human immunodeficiency virus (HIV) infection and/or neonate state [7,8]. However, there are reports of *S. boulardii* fungemia in critically ill patients with no obvious immunosuppression [9,10].

The source of the fungemia is thought to be the digestive tract, as has been documented in experimental studies for *C. albicans* [20]. Enteral translocation of ingested *S. boulardii* appears to be the main portal of entry into the bloodstream, especially in patients receiving high doses of the yeast along with antibiotics anaerobic activity [21]. This route was confirmed by light and scanning electron microscopy

Table 3 Concentration of yeasts in stool cultures of mice receiving *Saccharomyces boulardii* for 2 weeks and subsequently *Candida albicans* containing chow for 2 weeks (Group 4).

	Median yeast concentration (range) ($\log_{10}$ CFU/g of stool)				
	Last day of probiotic course	1 week after probiotic d/c[a]	2 weeks after probiotic d/c	3 weeks after probiotic d/c	4 weeks after probiotic d/c
S. boulardii	5.1 (4.9–5.4)	2.0 (0.0–2.1)	0[b]	0	0
C. albicans	-	7.8 (7.6–7.95)	6.7 (6.6–6.8)	4.5 (4.3–4.7)	4.25 (4.1–4.3)

[a]d/c: discontinuation.

[b]Values of 0 indicate that cultures did not reveal yeasts in the stools.

There was a statistically significant decline in median *C. albicans* CFU starting at 3 weeks after d/c of probiotic treatment (columns 4 and 5) when compared to median *C. albicans* CFU 1 week after d/c of probiotic treatment (column 2), ($P < 0.0001$).

Table 4 Concentration of yeasts in stool cultures of mice receiving *Saccharomyces boulardii* for 2 weeks and subsequently a combination of *S. boulardii* and *Candida albicans* containing chow for an additional 2 weeks (Group 5).

	Median yeast concentration (range) (log_{10} CFU/g of stool)				
	2 weeks after probiotic ini.[a]	3 weeks after probiotic ini.	4 weeks after probiotic ini.	5 weeks after probiotic ini.	6 weeks after probiotic ini.
S. boulardii	5.1 (4.9–5.4)	5.7 (5.6–5.9)	7.2 (6.9–7.5)	0.0 (0.0–2.0)	0[b]
C. albicans	-	7.85 (7.6–7.9)	5.5 (5.3–5.6)	4.4 (4.3–4.7)	4.2 (4.1–4.3)

[a]ini: initiation.
[b]Values of 0 indicate that cultures did not reveal yeasts in the stools.

examination of specimens prepared from the intestine of BALB/c mice given the yeast by intubation at a dosage of 1.5 g/kg every 6 h for up to 48 h [13]. In addition, translocation of *S. boulardii* to mesenteric lymph nodes, and rarely to the liver and spleen has been observed in immunosuppressed mice treated with excessively high yeast dose (10 mg) [14].

We have described a model of GI colonization by *C. albicans* in healthy adult mice that mimics the same process in humans [11,22,23]. Based on this experience we used a similar model to study the effects on GI colonization of three different *S. boulardii* dose regimens given per os to mice. We observed that the higher the dose, the greater the intestinal concentration of *S. boulardii* but this GI colonization was not sustained. Dissemination of the yeast to internal organs did not occur, despite administration of high doses of the probiotic. This is most probably due to the fact that the mice in the present experiments were young and healthy and the doses were not excessively high as described by other authors [13,14]. Most cases of dissemination have been observed in immunocompromised or critically ill individuals [6–9].

The present experiments were the first to evaluate the role of *S. boulardii* in healthy young mice. Immunosuppressed animals were not used since the purpose of the study was to investigate the virulence and potential dissemination of the yeast when given as probiotic, in healthy hosts. Furthermore, the data from the study is to be used as the basis for comparison in future studies with immunocompromised animals.

Previous studies in animals have demonstrated that *S. boulardii* reaches a steady-state gut concentration quickly and maintains a high stable level as long as the yeast is taken daily. Once administration is discontinued, *S. boulardii* is quickly eliminated from the gut [11,12]. It has also been confirmed that the yeast is located in the intestinal lumen and is not associated with the mucosa [12]. Orally administered *S. boulardii* does not permanently colonize the intestinal tract of mice and does not alter the faecal flora [12].

Similar findings were reported when *S. boulardii* was used in healthy volunteers [24,25]. It is of note that no morphological alterations of the human intestinal mucosa have been observed after treatment with high doses of lyophilized *S. boulardii* (250 mg four times per day) for 2 weeks [26].

The present experiments have also shown that *S. boulardii* at the doses given does not prevent murine intestinal colonization by *C. albicans* in healthy young adult mice. These findings confirm previous reports describing that in monoxenic mice harbouring *S. boulardii*, *C. albicans* became established at a level equivalent to that observed in mice harbouring *C. albicans* alone [27]. Similarly other investigators demonstrated that *S. boulardii* did not decrease GI population levels of *C. albicans*, *Clostridium difficile, Salmonella typhimurium or Shigella flexneri* in experimental mouse models [28–30]. However, in contrast to these previous reports, *S. boulardii* has been shown to antagonize *C. albicans* in gnotobiotic mice and in mice with chemically-induced colitis [27,31], as well as *E. coli* in healthy volunteers [32].

In conclusion, our data suggest that *S. boulardii* induces a substantial increase in the intestinal yeast concentration, when given by mouth in high doses. However, these increases are not sustained and not associated with dissemination of the yeast to internal organs in healthy young adult mice. Additionally, *S. boulardii* given in commonly used dosage does not prevent GI murine colonization by *C. albicans*.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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