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Kinetics of *Saccharomyces cerevisiae* Elimination from the Intestines of Human Volunteers and Effect of This Yeast on Resistance to Microbial Colonization in Gnotobiotic Mice

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When healthy volunteers were given a daily dose of 3×10^8 life-dehydrated *Saccharomyces cerevisiae* cells for 5 days, the volunteers excreted 10^5 living yeast cells per g of feces at first, but the yeast cells disappeared within 5 days of the end of treatment. In gnotobiotic mice, *S. cerevisiae* administered alone colonized the intestinal tract but did not interfere with previous or subsequent colonization by a variety of potentially enteropathogenic microorganisms. When these microorganisms were present, the intestinal counts of *S. cerevisiae* were greatly reduced.

Saccharomyces cerevisiae is an ascosporogenous yeast which has been successfully used in enzyme substitution therapy for congenital sucrase-isomaltase deficiency (2, 13). In Western Europe, it is commercially available as a combination with activated charcoal (Laboratories UCB, Nanterre, France) for the treatment of functional disorders of the intestine such as colitis or diarrhea. Although the basis of this therapy was explained in a laboratory report on the nonspecific stimulation of phagocytosis by *S. cerevisiae* (16), no controlled clinical trials of this treatment have been published. More-abundant data have been published on the effect of another yeast, *Saccharomyces boulardii*. The kinetics of intestinal elimination of this type of yeast have been studied in volunteers (4), and administration of *S. boulardii* has been shown to inhibit the in vitro growth of various microorganisms (5), increase resistance to colonization by *Candida* spp. (10), and reduce *Clostridium difficile*-induced mortality in experimentally infected rodents (8, 19). Although few authors have studied the antidiarrheal mechanism of action of *S. boulardii* (6, 20), the results of several investigations, including a prospective placebo-controlled study, suggest that *S. boulardii* may reduce diarrhea in various clinical situations (7, 11, 14, 17, 18).

In order to study whether similar results could be obtained with *S. cerevisiae*, we determined the kinetics of the elimination of *S. cerevisiae* after its oral absorption by healthy volunteers and the effect of this yeast on resistance to colonization in gnotobiotic mice.

Human volunteers. Six healthy, fully informed adult volunteers were included in the study. None had taken antibiotics for at least 1 month before the study started. For 5 days, volunteers were given three capsules a day, each containing 10^8 life-dehydrated cells of *S. cerevisiae* and 109 mg of activated charcoal (Laboratoires UCB). Each volunteer took 10^{10} spores of *Bacillus subtilis* with the last capsule as a transit marker (9). Freshly passed fecal samples were obtained once a day before and during the treatment and 1 week after treatment had ended.

Gnotobiotic mice. Adult germfree C3H mice (Centre de Sélection des Animaux de Laboratoire, Orléans, France) were maintained in plastic Trexler-type isolators. They were fed a locally prepared diet sterilized by gamma radiation (1). Autoclaved drinking water at pH 3 was given ad libitum. When necessary, *S. cerevisiae* was added to the drinking water at a final concentration of 10^{10} CFU of *S. cerevisiae* per liter. This water was changed every 2 days. The yeast cells in the drinking water were counted daily.

Some of the mice were inoculated once intragastrically with 10^8 CFU of *S. cerevisiae* and then challenged by intragastric gavage 5 days later with 10^8 CFU of one of five potentially pathogenic bacterial strains: enterotoxigenic *Escherichia coli* H10407 (from J. P. Craig), *Staphylococcus aureus* IGR4310, *Vibrio parahaemolyticus* J525C, *Vibrio cholerae* 569B (from J. P. Craig), or *C. difficile* IGR786. The rest of the mice were first challenged with one of these strains and then continuously treated with *S. cerevisiae* from day 5 on. All mice were killed 10 days after the first inoculation. Each mouse's intestine from pylorus to rectum was then removed, and the jejunum, ileum, and cecum were separated, individually weighed, and ground with an Ultra-Turrax grinder (Bioblock, Illkirch, France). *S. cerevisiae* and the associated bacteria then were counted in each anatomical segment.

Bacterial counts. Tenfold dilutions of the human fecal samples, mouse pellets, and mouse intestinal segments were spread on various types of agar and cultured for counts of five microbial strains, as follows: members of the family *Enterobacteriaceae*, 24 h at 37°C on Drigalski agar (Diagnostic Pasteur); enterococci, 48 h at 37°C on bile esculin agar (Difco); anaerobes, 48 h at 37°C on blood agar (BBL GasPak, Biomérieux, Charbonnières-les-Bains, France); *S. cerevisiae*, 72 h at 30°C on Sabouraud agar with 0.4 mg of gentamicin per ml; and *Bacillus thermophilus*, 24 h at 55°C on Mueller-Hinton agar.

Statistical analysis. Bacterial counts were converted into common logarithms, since the log distribution of intestinal bacterial counts has been shown to be normal (3). Student's *t* test was used to compare mean values.

Scanning electron microscopy. Mice were injected intraperitoneally with 2 mg of 1% ketamine solution (100 mg/kg of body weight; Parke-Davis, Courbevoie, France) and with 1

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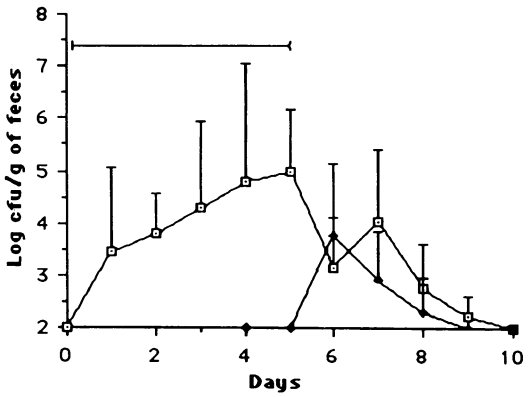

FIG. 1. Mean fecal counts of *S. cerevisiae* (⊡) and *B. thermophilus* (◆) in six human volunteers treated with 3×10^8 CFU of *S. cerevisiae* per day for 5 days (day 0 to day 5) and with one dose of 10^{10} CFU of *B. thermophilus* taken with the last dose of *S. cerevisiae*. Horizontal bar indicates duration of *S. cerevisiae* treatment.

mg of 0.5% acepromazine maleate acide solution (50 mg/kg of weight; Sanofi, Libourne, France). When no reflex-type reaction was obtained, the mice were injected by intracardiac perfusion with 0.4% glutaraldehyde solution. A small incision was made in the atrium to allow blood and fixative solution to leak out. When tissues were fixed, gut samples were taken and fixed, first in 0.4% glutaraldehyde for 15 min and then in 2% osmium tetroxide for 1 h. The day before microscopic observation, the samples were dehydrated in graded ethanols at concentrations of 50 to 100%, critical point dried, and sputter coated with gold palladium (12). Gut tissue preparations were examined with a JEOL 840A scanning electron microscope (JEOL, Paris, France).

In the volunteers, fecal counts of *S. cerevisiae* gradually rose during treatment to a maximum of about 10^5 CFU/g of feces and decreased immediately after the end of treatment at a rate equivalent to that of the transit marker, *B. thermophilus* (Fig. 1). Fecal counts of *Enterobacteriaceae*, enterococci, and total anaerobes were not significantly modified by *S. cerevisiae* treatment (Table 1).

In monoassociated mice (i.e., mice treated with only one kind of bacterium), all bacterial strains were established at high population levels (Table 2). When *S. cerevisiae* was inoculated preventively, i.e., prior to inoculation of bacterial strain, and counted 9 days later, less than 10^3 CFU/g of feces was found for all the bacterial strains inoculated except *C. difficile*. By contrast, all the strains, including *C. difficile*, colonized the intestine of the pretreated mice at concentrations similar to those observed in the monoassociated mice. When *S. cerevisiae* was inoculated curatively, i.e., after the inoculation of bacterial strains, the counts for bacteria were unchanged but those for *S. cerevisiae* rose, ranging from 3.5 ± 0.2 CFU/g of feces for *E. coli* H10407 to 6.0 ± 0.8 for *V. cholerae* 569B.

Administration of *S. cerevisiae* did not significantly modify the jejunal, ileal, or cecal counts of any of the strains inoculated into the monoassociated mice (data not shown).

Scanning electron microscopy studies (micrographs not shown) showed that high concentrations of yeast cells were present at the surface of the ileum of mice monoassociated with *S. cerevisiae*. However, this did not prevent *V. parahaemolyticus* J525C from also being present on the mucosa when this organism was inoculated after *S. cerevisiae*. In addition, when *E. coli* H10407 was administered before *S. cerevisiae*, almost no yeast cells were seen on the mucosa.

Our data show that volunteers who ingested *S. cerevisiae* excreted significant numbers of living yeast organisms throughout the 5 days of its administration. Fecal concentrations decreased rapidly after the end of the treatment, suggesting that no permanent implantation or multiplication of *S. cerevisiae* occurred. This is very similar to what had been observed with *S. boulardii* (4), a closely related yeast which has been shown to increase intestinal resistance to colonization markedly in experimental models (5, 8, 10, 16, 19) and to prevent or reduce diarrhea in various clinical situations (7, 14, 17, 18).

To establish whether *S. cerevisiae* displays the same activity as *S. boulardii*, we studied the effects of *S. cerevisiae* on resistance to colonization by various enteropathogenic bacteria. For this purpose, we used gnotobiotic mice, as described previously (15). We found that, contrary to what others observed with *S. boulardii*, none of the test strains was eliminated by the administration of *S. cerevisiae*, whether the latter was given before or after the test strain. Given that *S. boulardii* and *S. cerevisiae* may be closely

TABLE 1. Fecal bacterial counts in six healthy human volunteers before, during, and after 5 days of oral treatment with *S. cerevisiae*

Microorganisms	Bacterial count[a]			
	Before treatment	During treatment[b]		After treatment
	Day 0	Day 2	Day 5	Day 10
Enterobacteriaceae	7.7 ± 0.7	6.8 ± 1.3	7.6 ± 1.2	7.7 ± 0.9
Enterococci	7.7 ± 1.0	6.8 ± 1.5	8.5 ± 0.2	6.5 ± 1.2
Total anaerobes	10.5 ± 0.5	10.8 ± 0.7	10.5 ± 0.9	10.5 ± 0.2

[a] Values are means ± standard deviations for $\log_{10}$ CFU per gram of feces.
[b] Volunteers were treated from day 1 to day 5.

TABLE 2. Effect of curative or preventive oral administration of *S. cerevisiae* on bacterial counts of gnotobiotic mice monoassociated with various enteropathogenic microorganisms

Strain	Administration of *S. cerevisiae*[a]	CFU/g of feces[b]	
		Test strain	*S. cerevisiae*
S. cerevisiae	Monoassociation	NA	7.2 ± 0.1
E. coli H10407	None	8.8 ± 0.1	NA
	Preventive	9.3 ± 0.2	2.3 ± 0.5
	Curative	9.1 ± 0.4	3.5 ± 0.2
S. aureus IGR4310	None	9.6 ± 0.2	NA
	Preventive	8.3 ± 0.3	3.1 ± 0.4
	Curative	9.3 ± 0.2	5.9 ± 0.4
V. parahaemolyticus J525C	None	9.0 ± 0.3	NA
	Preventive	9.3 ± 0.2	2.5 ± 0.7
	Curative	8.9 ± 0.6	5.8 ± 0.2
V. cholerae 569B	None	9.0 ± 0.5	NA
	Preventive	9.3 ± 0.2	2.7 ± 0.6
	Curative	8.2 ± 0.3	6.0 ± 0.8
C. difficile IGR786	None	8.9 ± 0.4	NA
	Preventive	9.6 ± 0.3	6.0 ± 0.7
	Curative	9.6 ± 0.2	5.5 ± 0.2

[a] *S. cerevisiae* was inoculated 5 days before (curative) or 5 days after (preventive) monoassociation of the mice with a test strain. Mice monoassociated with *S. cerevisiae* were used as controls.
[b] Values are means ± standard deviations for $\log_{10}$ CFU per gram of feces. Six mice per group. NA, not applicable.

related and that the test conditions were apparently not much different in the two studies, the differences observed may be more strain related than species related. Finally, when *S. cerevisiae* was administered first, its concentration decreased after administration of the test strains. Scanning electron microscopy pictures showed that when this occurred, very few yeast cells were associated with the intestinal mucosa.

Taken together, these results do not suggest that the alleged antidiarrheal effect of preparations containing *S. cerevisiae* is mediated by an increase in resistance to colonization by intestinal pathogens.

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