Variable biotherapeutic effects of *Lactobacillus acidophilus* isolates on orogastric and systemic candidiasis in immunodeficient mice

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Summary

Two commercially available isolates of *Lactobacillus acidophilus* (NCFM and LA-1) were compared for their capacities to protect immunodeficient *bg/bg-nu/nu* and *bg/bg-nu/+* mice from candidiasis. *L. acidophilus* NCFM prolonged survival of adult and neonatal *bg/bg-nu/nu* mice, inhibited disseminated candidiasis in both mouse strains, suppressed weight loss associated with *Candida albicans* infection in *bg/bg-nu/nu* females, but did not decrease the severity or the incidence of orogastric candidiasis in gnotobiotic mice. *L. acidophilus* LA-1 suppressed numbers of *C. albicans* in the alimentary tracts of *bg/bg-nu/+* mice and reduced the severity of mucosal candidiasis in *bg/bg-nu/nu* and *bg/bg-nu/+* mice; however, *L. acidophilus* LA-1 did not improve the survival of *bg/bg-nu/nu* mice after oral challenge (colonization) with *C. albicans* and it was associated with lethality in gnotobiotic adult female *bg/bg-nu/nu* mice. These results demonstrate that the two isolates of *L. acidophilus* differed in their capacity to protect immunodeficient mice from candidiasis.

Key words Probiotics, *Lactobacillus*, candidiasis, immunodeficient mice

Efectos bioterapéuticos variables de aislamientos de *Lactobacillus acidophilus* en las candidiasis orogástrica y sistémica en ratones inmunodeficientes

Resumen

Se comparó la capacidad de dos aislamientos comerciales de *Lactobacillus acidophilus* (NCFM and LA-1) para proteger a ratones inmunodeficientes *bg/bg-nu/nu* y *bg/bg-nu/+* frente a la candidiasis. *L. acidophilus* NCFM prolongó la supervivencia de ratones *bg/bg-nu/nu* adultos y neonatos, inhibió la candidiasis diseminada en ambas cepas murinas, suprimió la pérdida de peso asociada con la infección por *Candida albicans* en hembras *bg/bg-nu/nu*, pero no redujo la severidad o la incidencia de candidiasis orogástrica en ratones gnotobióticos. *L. acidophilus* LA-1 suprimió el número de *C. albicans* en los tractos alimentarios de los ratones *bg/bg-nu/+* y disminuyó la severidad de la candidiasis mucosa en los ratones *bg/bg-nu/nu* y *bg/bg-nu/+*. Sin embargo, *L. acidophilus* LA-1 no mejoró la superviviencia de los ratones *bg/bg-nu/nu* tras inoculación oral con *C. albicans* (colonización) y se asoció con letalidad en hembras gnotobióticas adultas de ratones *bg/bg-nu/nu*. Estos resultados demuestran que los aislamientos de *L. acidophilus* difieren en su capacidad para proteger a ratones inmunodeficientes frente a la candidiasis.

Palabras clave Probióticos, *Lactobacillus*, Candidiasis, Ratones inmunodeficientes

Several *Lactobacillus* species are currently used as probiotics, i.e., live bacteria used as food supplements to provide beneficial effects to the host. Probiotic bacteria can produce bacteriocin-like compounds that inhibit infectious microorganisms [1], they can adhere to the epithelium of the alimentary tract and block adherence of pathogens [2,3] and they can stimulate host defense mechanisms [4-6].

Lactobacillus acidophilus is an important probiotic species, which has been reported to protect humans from

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Aceptado para publicación el 24 de septiembre de 1998

Candida albicans vaginitis [7], and immunodeficient mice from orogastric candidiasis [6]. In contrast, *L. acidophilus* did not protect immunosuppressed mice from systemic candidiasis [8], or protect children from enterotoxigenic *Escherichia coli* diarrhea [9]. The disparity of the reports for and against protective effects by *L. acidophilus* may be due to strain differences that manifest under *in vitro* and *in vivo* growth conditions [10].

The purpose of this study was to determine if *L. acidophilus* isolates from two different commercial sources, provided similar protection against orogastric and systemic candidiasis in a *C. albicans*-susceptible immunodeficient mouse model.

MATERIALS AND METHODS

Microorganisms. Commercial starter cultures of probiotic bacteria were obtained from the following sources: *L. acidophilus* NCFM was obtained from Rhône-Poulenc, Inc. of Madison, WI. The *L. acidophilus* LA-1 was provided by Chr. Hansen's Laboratory, Inc., Milwaukee, WI. Bacteria were grown overnight in deMan, Rogosa, Sharpe (MRS) medium (Difco, USA) or on plates of MRS medium with 1.5% agar in anaerobe jars (Gaspack; BBL, USA) containing anaerobic generators (AnaeroPack System; Carr-Scarborough Microbiologics, USA) at 37°C. *C. albicans* was cultured on Sabouraud's dextrose agar (SDA; BBL, Becton-Dickenson Microbiology Systems, USA) at 37°C. Microbiological identification was verified using the API 50CH biochemical identification system (BioMérieux-Vitek,USA) and cellular fatty acid chromatography (MIDI, Inc., USA).

Mice. C57BL/6 *bg/bg-nu/nu* and *bg/bg-nu/+* mice were obtained from breeding stocks maintained at the University of Wisconsin Gnotobiote Laboratory, Madison, WI (http://www.biostat.wisc.edu/gnotolab/gnotolab.html). Ten germfree male *bg/bg-nu/nu* and female *bg/bg-nu/+* mice were mated to obtain litters with approximately equal numbers of nude and heterozygous mice. Five groups of breeder mice, their progeny, and all adult mice were housed in sterile flexible film isolators and colonized with pure cultures of either *C. albicans* or one of the *L. acidophilus* isolates by swabbing their oral and anal orifices with a culture that contained 1 x 10^7 CFU/ml of the microbe. Mice colonized with either *L. acidophilus* isolate for 2 weeks were orally inoculated with cultures of *C. albicans* (swabbed orally with 1 x 10^7 CFU/ml *C. albicans*) for assessment of the effects of probiotics on colonization and infection by *C. albicans*. Microbial colonizations were monitored with quantitative cultures of homogenized and serially diluted feces collected from mice housed in the gnotobiotic isolators. Dilutions of homogenized feces were made on SDA and incubated aerobically at 37°C for *C. albicans*, or on MRS agar incubated anaerobically (AnaeroPack) at 37°C for *L. acidophilus*. All mice were given autoclave-sterilized food, water, and bedding, *ad libitum*. Bacterial and fungal cultures were carried out weekly to verify the microbial integrity of the experiment.

Survival and growth of immunodeficient mice. Survival of mice born to germfree or gnotobiotic mothers was assessed at 4 and 8 weeks of age. Survival of *L. acidophilus* (NCFM or LA-1)-colonized adult mice was assessed at 4 and 8 weeks after oral colonization with *C. albicans*.

Body weights were measured on a Sartorius balance (Brinkman Instruments, USA). Body weights of adult mice and growth rates of newborn mice between 4 and 8 weeks of age were compared with weights of germfree control mice.

Alimentary tract colonization. Mice were colonized two weeks with *L. acidophilus* NCFM or LA-1 before oral challenge with *C. albicans*. *L. acidophilus* and *C. albicans* colonization of the alimentary tracts of mice was assayed by counting colonies of viable microbes (CFU) recovered from feces and the contents of the stomachs, small and large intestines, and ceca of euthanized mice. Contents were washed out of the intestines with sterile water, serially diluted, and 50 µl aliquots were inoculated onto SDA and MRS agar plates. The MRS plates were incubated anaerobically and the SDA plates were incubated aerobically at 37°C. A 1 ml aliquot of each 5 ml suspension of intestinal contents was dried overnight in a tared aluminum weighing dish at 80°C. The dried dishes were cooled to room temperature and weighed. The number of viable *C. albicans* or *L. acidophilus* are reported as CFU/g (dry weight) of intestinal contents or feces.

Systemic candidiasis of endogenous origin. The spleens, livers, and kidneys were aseptically excised, homogenized in glass tissue grinders with 5 ml sterile distilled water, serially diluted, and cultured on SDA or anaerobic MRS agar to assess systemic dissemination of *C. albicans* and the bacteria in the gnotobiotic mice euthanized at different time intervals after colonization. The number of *C. albicans* or bacteria in the internal organs are reported as CFU/g (dry weight) tissue.

Histological evaluation. The alimentary tracts and major internal organs of the mice were fixed in 10% formaldehyde in PBS. The fixed tissues were dissected, embedded in paraffin, and sectioned onto slides for staining with hematoxylin and eosin stains, Gomori's methenamine silver stain for fungi, and a Gram stain. Tissue sections from several areas of the alimentary tracts and the major internal organs were evaluated by a pathologist for evidence of infection by the following criteria: Histopathology score in infected tissues – 0, no infection evident; 1, 1-10 microorganisms per high power field (HPF, 400X); 2, 10-50 microorganisms per HPF; 3, 50-100 microorganisms per HPF (yeast and hyphae of *C. albicans*); 4, confluent microorganisms per HPF (yeast and hyphae of *C. albicans*), and 5, confluent microorganisms per HPF with hyphal penetration of viable tissues (yeast and hyphae of *C. albicans*). Photomicrographs were produced with a Nikon Optiphot microscope (Nikon Inc., USA) equipped with a Nikon DX-100M automatic camera and a Sony CCD camera attached to a Targa frame grabber (Truevision, Inc.,USA) using Image Pro Plus imaging software (Media Cybernetics, USA).

Immune response to C. albicans and L. acidophilus. Serum immunoglobulin (IgG, IgA, and IgM) concentrations were determined with commercial radial immunodiffusion assays (The Binding Site, USA).

Statistical analyses. Repeated Measures Analysis of Variance (ANOVA) or the Student t test was implemented on log transformed data to assess the significance of differences in numbers of viable *C. albicans* or *L. acidophilus* in the alimentary tracts and internal organs of mice colonized with probiotic bacteria and/or *C. albicans* [11]. The ANOVA was also employed to detect significant differences in body weights (two-tailed analysis to evaluate enhanced or inhibited growth) of probiotic-colonized adult and neonatal mice and to assess significant differences between histopathology severity scores from tissue sections of mice with mucosal candidiasis that were euthanized at different time intervals after colonization with *C. albicans*.

RESULTS

Suppression of C. albicans in the alimentary tract. Numbers of *L. acidophilus* NCFM or LA-1 in feces of the mice remained relatively constant over the course of the experiments (e.g., average of 9.5 ± 0.2 and 9.9 ± 0.5 log10 CFU/g (dry weight) of *L. acidophilus* NCFM in *bg/bg-nu/nu* and *bg/bg-nu/+* mice, respectively). The average numbers of *L. acidophilus* LA-1 in the feces of *bg/bg-nu/nu* and *bg/bg-nu/+* mice over the course of the experiments were respectively, 9.7 ± 0.1 and 9.3 ± 0.1 log10 CFU/g (dry weight). The levels of colonization by either strain of bacteria were not significantly different and were not significantly altered by diassociation with *C. albicans* in either strain of immunodeficient mice.

Table 1 shows that in *bg/bg-nu/nu* mice, *L. acidophilus* NCFM suppressed the numbers of viable *C. albicans* (in comparison to *C. albicans*-monoassociated controls) in the stomachs and small intestines. *L. acidophilus* LA-1 also suppressed *C. albicans* in the small intestines, colons, and feces of *bg/bg-nu/nu* mice (Table 1). In euthymic *bg/bg-nu/+* mice, *L. acidophilus* LA-1 significantly inhibited *C. albicans* throughout the alimentary tract and in feces by as much as 100-fold compared with *C. albicans*-monoassociated mice (Table 1). Neither *C. albicans* nor either of the two probiotic bacteria were eliminated from the alimentary tracts of the mice over the 12-week study.

Table 1. *L. acidophilus* NCFM and LA-1 inhibit *C. albicans* in the alimentary tracts of gnotobiotic mice.

Microbial status	No. of viable *C. albicans*[a]									
	bg/bg-nu/nu mice					*bg/bg-nu/+* mice				
	Stomach	Sm. Int.	Cecum	Colon	Feces	Stomach	Sm. Int.	Cecum	Colon	Feces
C. albicans alone	8.8 ± 0.2	9.1 ± 0.2	9.4 ± 0.0	9.1 ± 0.4	9.8 ± 0.1	8.4 ± 0.2	8.1 ± 0.3	9.1 ± 0.2	8.3 ± 0.2	8.6 ± 0.2
C. albicans plus:										
L. acidophilus NCFMb	7.8 ± 0.3[c]	7.4 ± 0.4[c]	9.0 ± 0.2	7.8 ± 0.3	9.8 ± 0.1	7.9 ± 0.2	7.5 ± 0.2	8.8 ± 0.1	7.8 ± 0.2	8.5 ± 0.1
L. acidophilus LA-1	8.3 ± 0.1	8.1 ± 1.0[c]	9.1 ± 0.4	7.7 ± 0.1[c]	8.9 ± 0.1[c]	6.0 ± 0.3[cd]	6.4 ± 0.4[cd]	6.9 ± 0.4[d]	6.5 ± 0.4[cd]	8.4 ± 0.1

a: Mean ± SEM log10 CFU/g (dry wt.) of *C. albicans* isolated from intestinal contents from 4 to 21 mice/group at 4 to 12 weeks after colonization.
b: Results for *L. acidophilus* NCFM have been reported previously [6].
c: Significantly fewer *C. albicans* than the *C. albicans*-monoassociated mice, P < 0.05 by ANOVA.
d: Significantly fewer *C. albicans* were present with *L. acidophilus* LA-1 than with *L. acidophilus* NCFM, P < 0.05 by ANOVA.

Probiotic inhibition of systemic candidiasis. Compared to *C. albicans* dissemination in gnotobiotic mice colonized with only *C. albicans* (75% dissemination in *bg/bg-nu/nu* mice and 36% dissemination in *bg/bg-nu/+* mice), the presence of either *L. acidophilus* NCFM or LA-1 in the alimentary tracts reduced the incidence of disseminated candidiasis in both mouse strains (Table 2). *L. acidophilus* NCFM protected *bg/bg-nu/nu* mice from disseminated candidiasis of endogenous origin better than *L. acidophilus* LA-1 (Table 2).

Table 2. Inhibition of systemic candidiasis by *L. acidophilus* NCFM or LA-1.

Microbial status	*bg/bg-nu/nu* mice		*bg/bg-nu/+* mice	
	incidence[a] (%)	No. of *C. albicans*[b]	incidence[a] (%)	No. of *C. albicans*[b]
C. albicans	75	7.0 ± 0.1	36	6.8 ± 1.2
C. albicans plus:				
L. acidophilus NCFMc	12	10	0	0
L. acidophilus LA-1	55	6.0 ± 1.3	25	2.7

a: % incidence = % mice with disseminated candidiasis, 4 to 27 mice per group.
b: No. of *C. albicans* = Mean ± SEM log10 CFU *C. albicans*/g homogenized tissue (dry weight).
c: Results for *L. acidophilus* NCFM have been reported previously [6].

Histological examination of tissues from probiotic-colonized mice. Orogastric candidiasis, defined as the presence of *C. albicans* in the keratinized mucosal epithelia, was not prevented by prior colonization of either strain of mice with *L. acidophilus* NCFM or LA-1. In comparison to mice monoassociated with *C. albicans*, the severity of orogastric candidiasis in *bg/bg-nu/nu* mice colonized with *C. albicans* and *L. acidophilus* NCFM or *C. albicans* and *L. acidophilus* LA-1 was not significantly reduced, although the mice survived longer than *C. albicans*-monoassociated mice (Table 3).

Table 3. Histopathology of gastric candidiasis in mice diassociated with *C. albicans* and *L. acidophilus* NCFM or LA-1.

Microbial status`	Average histopathology severity score (# mice)[a]					
	bg/bg-nu/nu			bg/bg-nu+		
	1-2 wk	3-5 wk	6-8 wk	1-2 wk	3-5 wk	6-8 wk
C.albicans	3 (11)	3 (6)	ND[b]	2 (37)	3 (12)	4 (17)
C. albicans plus:						
L. acidophilus NCFM[c]	3 (7)	3 (2)	5 (2)	3 (6)	2 (3)	1 (6)
L. acidophilus LA-1	d	2 (7)	3 (2)	ND[d]	1 (6)	3 (8)

a : Numbers in parentheses represent the numbers of mice with the indicated histopathology scores. Histopathology scores are graded (1 to 5); see Materials and Methods
b : Mice died before time point.
c : Results for *L. acidophilus* have been reported previously [6].
d : No samples available at this time point.

Although the incidence and severity of orogastric *C. albicans* infections in *bg/bg-nu/nu* mice was not decreased by *L. acidophilus* NCFM or LA-1, the percentage of these mice with obvious inflammation at sites of *C. albicans* infection was increased by *L. acidophilus* NCFM. Inflammation was observed in stomachs of 30% of *C. albicans*-monoassociated *bg/bg-nu/nu* mice; however, it was evident in 71% of mice diassociated with *C. albicans* and *L. acidophilus* NCFM. The *bg/bg-nu/nu* mice colonized with *L. acidophilus* LA-1 and *C. albicans* had no observable increase in gastric inflammation (25%), in comparison to *C. albicans*-monoassociated controls.

Survival of mice colonized with L. acidophilus *NCFM or LA-1 and* C. albicans. Adult and newborn *bg/bg-nu/nu* mice die within several weeks after they are colonized with *C. albicans*. Adult *bg/bg-nu/nu* mice colonized with *L. acidophilus* NCFM, but not those colonized with *L. acidophilus* LA-1, survived longer after oral challenge with *C. albicans* than mice colonized with a pure culture (monoassociated) of *C. albicans* (Table 4). Survival of neonatal *bg/bg-nu/nu* mice born to breeders diassociated with either isolate of *L. acidophilus* and *C. albicans* was prolonged, compared with neonatal mice born to breeders monoassociated with *C. albicans*. *L. acidophilus* NCFM provided better protection (prolonged the survival) of neonatal mice than *L. acidophilus* LA-1 (Table 4).

Effects of probiotics and C. albicans *on adult body weights.* Adult *bg/bg-nu/nu* and *bg/bg-nu/+* mice monoassociated for 4 to 12 weeks with *L. acidophilus* NCFM had smaller body weights than their germfree counterparts. In contrast, *bg/bg-nu/nu* and *bg/bg-nu/+* mice monoassocia-

Table 4. Protection of immunodeficient mice from lethal candidiasis by *L. acidophilus* NCFM or LA-1.

	% Survival (no. mice/group)			
	bg/bg-nu/nu		*bg/bg-nu/+*	
Microbial status	4 wk	8-12 wk	4 wk	8-12 wk
Adult mice				
C. albicans	50 (14)	0 (7)[a]	100 (24)	100 (24)
C. albicans plus:				
L. acidophilus NCFM[b]	100 (8)[c]	100 (8)[c]	100 (6)	100 (6)
L. acidophilus LA-1	20 (17)	11 (9)	100 (19)	100 (16)
Newborn mice				
C. albicans	0 (15)	ND[D]	82 (13)	100 (11)
C. albicans plus:				
L. acidophilus NCFM[b]	70 (25)[c]	50 (16)	100 (28)	100 (18)
L. acidophilus LA-1	52 (29)[c]	0 (9)[a]	100 (23)	100 (10)

a: The mice died at 4 to 5 weeks of age.
b: Results for *L. acidophilus* NCFM have been reported previously [6].
c: Significantly increased survival compared to *C. albicans*-monoassociated control, P < 0.05 by ANOVA and the Rank Sum test.
d: No data because of early deaths.

Table 5. Effects of *L. acidophilus* NCFM or LA-1 on *C. albicans*-induced body weight loss of adult mice.

	Mean ± SEM body weight (g)			
	bg/bg-nu/nu		*bg/bg-nu/+*	
Microbial status	Male	Female	Male	Female
Germfree	32.6 ± 2.3	24.8 ± 0.5	32.7 ± 0.1	28.5 ± 1.0
L. acidophilus NCFM[a]	19.6 ± 0.8[b]	17.0 (1)	24.7 (1)	21.8 ± 0.4[a]
L. acidophilus LA-1	30.3 ± 0.4	26.5 ± 0.5[c]	34.7 ± 0.7[c]	34.9 ± 2.2[b]
C. albicans	18.4 ± 2.5	15.2 ± 0.3	31.1 ± 0.6	29.9 ± 3.0
C. albicans plus:				
L. acidophilus NCFM[a]	19.0 ± 3.0	18.1 ± 1.0c	30.1 ± 0.4	29.7 ± 1.1
L. acidophilus LA-1	26.0 ± 3.0	17.4 ± 2.6	24.1 ± 0.7	34.8 ± 2.3

Mice were colonized 4 to 12 weeks with *C. albicans*. There were 3 to 11 mice/group unless otherwise noted by the number in parentheses ( ).
a: Results for *L. acidophilus* NCFM have been reported previously [6,15].
b: Significantly less than the germfree control, P < 0.05 by ANOVA.
c: Significantly greater than the germfree control, P < 0.05 by ANOVA.

Table 6. Effect of *L. acidophilus* NCFM or LA-1 on *C. albicans*-induced inhibition of neonatal growth rates.

		Body wt at 4 wk age		Body wt at 8 wk age	
Microbial status	Mouse genotype	Male	Female	Male	Female
Germfree	*bg/bg-nu/nu*	18.8 ± 2.2	16.5 ± 1.6	25.8 ± 1.0	22.6 ± 0.05
	bg/bg-nu/+	23.8 ± 2.0	20.7 ± 1.3	30.3 ± 0.9	24.0 ± 0.8
L. acidophilus NCFM[a]	*bg/bg-nu/nu*	ND[a,b]	ND[b]	19.6 ± 0.8	19.7 ± 2.7
	bg/bg-nu/+	19.6 ± 1.0	14.9 ± 1.3[c]	27.8 ± 0.7	22.5 ± 0.7
L. acidophilus LA-1	*bg/bg-nu/nu*	13.0 ± 0.7[c]	10.5 ± 0.5[c]	25.9 ± 0.4[b]	21.3 ± 0.5
	bg/bg-nu/+	20.5 ± 0.5	15.2 ± 0.6[d]	28.4 ± 0.6	22.5 ± 0.7
C. albicans	*bg/bg-nu/+*	7.1 ± 0.6	21.7 ± 2.9	11.7 ± 1.1	19.4 ± 0.5
C. albicans plus:					
L. acidophilus NCFM[a]	*bg/bg-nu/+*	13.0 ± 0.4[c,e]	27.3 ± 0.6[e]	15.1 ± 0.9[c]	22.6 ± 1.2
L. acidophilus LA-1	*bg/bg-nu/+*	15.3 ± 2.1[c,e]	25.8 ± 0.7[e]	12.8 ± 0.7[c]	19.7 ± 0.5[c]

Experimental results from 3 to 24 mice/group.
a: Results for *L. acidophilus* NCFM have been reported previously [6,15].
b: ND, not done.
c: Significantly smaller body weights than germfree controls, P < 0.05 by ANOVA.
d: Significant differences were observed in results from the two isolates of *L. acidophilus*, P < 0.05 by ANOVA.
e: Significantly greater body weights than *C. albicans*-monoassociated mice, P < 0.05, by ANOVA.

ted with *L. acidophilus* LA-1 had body weights equal to or greater than germfree controls (Table 5).

Adult *bg/bg-nu/nu* mice that survived monoassociation with *C. albicans* for 4 to 12 weeks had lower body weights than germfree control mice (Table 5). Adult *bg/bg-nu/+* mice diassociated with *C. albicans* and *L. acidophilus* NCFM or *L. acidophilus* LA-1 were also significantly smaller than their germfree counterparts (Table 5).

Effects of probiotics and C. albicans *on growth of neonatal mice.* Insufficient numbers of *bg/bg-nu/nu* pups were born to breeders monoassociated with *L. acidophilus* NCFM to obtain body weights from 4-week-old mice. Male and female *bg/bg-nu/nu* and *bg/bg-nu/+* pups colonized with *L. acidophilus* LA-1 were significantly smaller than germfree controls at 4 weeks of age (Table 6). Due to the early deaths of *bg/bg-nu/nu* mice born to breeders monoassociated with a pure culture of *C. albicans*, comparisons of their body weights with the weights of pups born to mice diassociated with *C. albicans* and probiotics could not be made. The body weights of male *bg/bg-nu/+* pups at 4 and 8 weeks of age and diassociated with *L. acidophilus* NCFM or LA-1 and *C. albicans* were significantly larger than mice born to mothers monoassociated with *C. albicans*; however, they were smaller than the germfree controls (Table 6).

Immune responses. Compared with germfree mice, euthymic *bg/bg-nu/+* mice monoassociated with *L. acidophilus* NCFM or LA-1 had increased serum IgG and IgM (Table 7). Serum from *C. albicans*-monoassociated *bg/bg-nu/+* mice had more IgG, IgA, and IgM (although IgM induction was not statistically significant) than germfree mice (Table 7). In comparison to sera from germfree mice, IgM was increased in sera from *L. acidophilus* LA-1-monoassociated *bg/bg-nu/nu* mice. Although some *bg/bg-nu/nu* mice appeared to respond to *C. albicans* monoassociation with increased IgG, IgA, and IgM in sera, the increases were not statistically significant for all the mice in the experimental group (Table 7). Sera from *bg/bg-nu/+* mice that were diassociated with *L. acidophilus* NCFM or LA-1 and *C. albicans* had more IgG, IgA, and IgM than sera from germfree control mice, but less IgG, IgA, and IgM than sera from *C. albicans*-monoassociated mice. The latter data suggests that mice diassociated with *L. acidophilus* NCFM or LA-1 and *C. albicans* did not form as much antibody as *C. albicans*-monoassociated mice.

Table 7. Immunoglobulins in sera of mice colonized with *L. acidophilus* NCFM or LA-1, with or without *C. albicans*.

		Immunoglobulin in mouse sera (µg/ml)[a]		
Microbial status	Mouse genotype	IgG	IgA	IgM
Germfree	*bg/bg-nu/nu*	293 ± 51	< 200	28 ± 2
	bg/bg-nu/+	301 ± 123	< 200	26 ± 9
L. acidophilus NCFM[b]	*bg/bg-nu/nu*	ND	ND	ND
	bg/bg-nu/+	754 ± 39[c]	< 200	77 ± 11
L. acidophilus LA-1	*bg/bg-nu/nu*	281 ± 38	< 200	105 ± 8[c]
	bg/bg-nu/+	705 ± 192[c]	220 ± 20	152 ± 21[c]
C. albicans	*bg/bg-nu/nu*	1936 ± 1049	229 ± 29	32 ± 7
	bg/bg-nu/+	2257 ± 121[c]	894 ± 21[c]	54 ± 12
C. albicans plus:				
L. acidophilus NCFM[b]	*bg/bg-nu/nu*	244 ± 25	< 200	48 ± 24
	bg/bg-nu/+	1285 ± 292[c]	761 ± 75[c]	74 ± 5[cd]
L. acidophilus LA-1	*bg/bg-nu/nu*	1194 ± 349[cd]	< 200	167 ±121
	bg/bg-nu/+	1847 ± 350[c]	335 ± 119[cd]	31 ± 7[c]

a: Mean ± SEM, 5 mice per group, colonized 4 to 8 weeks. ND, not done.
b: Results for *L. acidophilus* NCFM have been reported previously [6,15].
c: Results were significantly greater than the germfree control, P < 0.05.
d: Results were significantly different between the two isolates of *L. acidophilus*, P < 0.05.
The limits of detection for IgA levels was 200 µg/ml.

DISCUSSION

Probiotic bacteria vary in their capacities to protect hosts from infectious diseases [12]. Inconsistencies in the prophylactic and biotherapeutic effects of probiotic bacteria have created an atmosphere of doubt about their efficacy. In this study, we tested the hypothesis that different isolates of the same species of probiotic bacteria would differ in their capacity to protect immunodeficient mice from orogastric (mucosal) candidiasis and systemic (disseminated) candidiasis of endogenous origin.

Our previous research indicated that *L. acidophilus* NCFM colonized the alimentary tracts of *bg/bg-nu/nu* mice and prolonged their survival after oral challenge with *C. albicans*, in comparison to *C. albicans*-monoassociated controls [6]. In this study, we compared the capacities of *L. acidophilus* NCFM and *L. acidophilus* LA-1 (isolates from different sources) to prolong the survival of immunodeficient *bg/bg-nu/nu* mice after oral challenge with *C. albicans*. Both *L. acidophilus* NCFM and LA-1 prolonged survival of *bg/bg-nu/nu* mice after colonization with *C. albicans* (compared to *C. albicans*-monoassociated mice), however *L. acidophilus* NCFM protected the mice (i.e., prolonged their survival) better than *L. acidophilus* LA-1.

An important attribute of probiotic bacteria is their capacity to reduce systemic infections of endogenous origin by enteric pathogens [13]. We previously observed that viable *L. acidophilus* NCFM inhibited the dissemination of *C. albicans* from the gut to internal organs in *bg/bg-nu/nu* and *bg/bg-nu/+* mice [6]. In this study, *L. acidophilus* NCFM and LA-1 decreased (compared with dissemination in *C. albicans*-monoassociated mice) the incidence of disseminated candidiasis; however, *L. acidophilus* NCFM protected against disseminated candidiasis better than *L. acidophilus* LA-1. With the gnotobiotic immunodeficient mouse models we used in this study, we were again able to show different *in vivo* probiotic effects of the two *L. acidophilus* isolates.

In a previous study [6] viable *L. acidophilus* NCFM was unable to protect *bg/bg-nu/nu* mice from orogastric candidiasis, as compared to *C. albicans*-monoassociated mice. In contrast to *C. albicans*-monoassociated controls, viable *L. acidophilus* NCFM was able to protect *bg/bg-nu/+* mice against orogastric candidiasis. In this study, *L. acidophilus* LA-1 reduced the severity of gastric candidiasis in *bg/bg-nu/nu* mice and slowed the progression of gastric candidiasis in *bg/bg-nu/+* mice. The less impressive survival of *bg/bg-nu/nu* mice that were colonized with *L. acidophilus* LA-1 and then orally challenged with *C. albicans*, as compared to the survival of mice colonized with *L. acidophilus* NCFM and orally challenged with *C. albicans* suggests that inhibition of gastric candidiasis did not protect these mice from lethal candidiasis. Interestingly, the current study shows that *L. acidophilus* NCFM protected the mice better from systemic candidiasis of endogenous origin than *L. acidophilus* LA-1; however, *L. acidophilus* LA-1 provided better protection from orogastric candidiasis than strain NCFM.

Another protective attribute of probiotic bacteria is their capacity to suppress viable *C. albicans* in the alimentary tracts of *bg/bg-nu/nu* and *bg/bg-nu/+* mice [6]. In this study, prior colonization with *L. acidophilus* NCFM was associated with significantly decreased numbers of viable *C. albicans* in the stomachs and small intestines, and prior colonization with *L. acidophilus* LA-1 also significantly decreased the numbers of *C. albicans* in the small and large intestines of *bg/bg-nu/nu* mice after oral *C. albicans* challenge, as compared with viable *C. albicans* in monoassociated control mice. The combination of decreased numbers of *C. albicans* in the alimentary tract and inhibited disseminated candidiasis of endogenous origin likely contributed to the prolonged survival of the *bg/bg-nu/nu* mice.

In a prior study, we reported that orogastric *C. albicans* infections in adult *bg/bg-nu/nu* mice induced weight loss and that some probiotic bacteria were able to prevent the weight loss [6]. In this study, *L. acidophilus* NCFM significantly attenuated the *C. albicans*-induced weight loss in adult female *bg/bg-nu/nu* mice; however, the mice still weighed less than germfree controls. The growth of newborn *bg/bg-nu/nu* and *bg/bg-nu/+* mice (i.e., born to *C. albicans*-monoassociated breeders) is also suppressed, in comparison to germfree controls [6]. In this study, the growth of male and female *bg/bg-nu/+* pups born to *L. acidophilus* NCFM or LA-1 and *C. albicans*-diassociated mice was significantly improved at 4 weeks of age compared to the growth of pups born to *C. albicans*-monoassociated mice.

Immunostimulation is considered to be a mechanism used by probiotic bacteria to protect hosts from infectious microorganisms [4,5,14]. We quantified immunoglobulin isotypes in sera to evaluate the capacity of *L. acidophilus* NCFM and LA-1 to stimulate antibody production in gnotobiotic mice. Quantitative analyses of immunoglobulin isotypes showed that both *L. acidophilus* strains stimulated production of IgG and IgM in *bg/bg-nu/+* mice. Monoassociation of *bg/bg-nu/+* mice with *C. albicans* significantly increased the amount of IgG and IgA in sera. After *L. acidophilus* NCFM- or LA-1-monoassociated mice were orally challenged with *C. albicans*, the concentrations of serum IgG and IgA were significantly lower than in the sera from *C. albicans*-monoassociated mice. The latter results suggest that the *L. acidophilus* isolates suppressed the antibody responses of mice to *C. albicans* antigens.

In this study, two isolates of *L. acidophilus* obtained from two different commercial sources were compared for their capacity to protect immunodeficient mice from orogastric and systemic candidiasis. Our data shows that the isolates (identified as the same species by *in vitro* biochemical tests) have different probiotic properties *in vivo*. *L. acidophilus* NCFM provided better protection of immunodeficient mice from systemic (disseminated) candidiasis of endogenous origin than *L. acidophilus* LA-1. Conversely, *L. acidophilus* LA-1 was better able to protect the mice from orogastric (mucosal) candidiasis than *L. acidophilus* NCFM. The overall conclusion from this study is that both *L. acidophilus* NCFM and LA-1 can protect immunodeficient mice from candidiasis, but probiotic strains can differ in the types and degree of biotherapeutic effects they can induce against orogastric and systemic candidiasis.

We wish to thank JoAnne Croft and Barbara Reese at the University of Wisconsin Gnotobiote Laboratory for assistance in the care of the mice used in this study. We also thank Donna Brackett for typing the manuscript. This work was supported by funds provided by the Ross Products Division of Abbott Laboratories, Columbus, OH.

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