Biotherapeutic effects of *Bifidobacterium* spp. on orogastric and systemic candidiasis in immunodeficient mice

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Summary

Two commercially available *Bifidobacterium* spp. (*Bifidobacterium infantis* and *Bifidobacterium lactis*) were compared for their capacities to protect immunodeficient *bg/bg-nu/nu* and *bg/bg-nu/+* mice from orogastric and lethal candidiasis. Both *Bifidobacterium* spp. prolonged the survival of *Candida albicans*-colonized adult and neonatal *bg/bg-nu/nu* mice. The bifidobacteria affected the production of antibodies to *C. albicans*, inhibited disseminated candidiasis, suppressed weight loss associated with *C. albicans* infection, inhibited the growth of *C. albicans* in the alimentary tract, inhibited systemic candidiasis of endogenous origin, and decreased the severity of gastric candidiasis in both mouse strains. *B. infantis* inhibited systemic candidiasis of endogenous origin better than *B. lactis*; however, *B. lactis* was significantly more effective at inhibiting *C. albicans* colonization of the alimentary tract, suppressing gastric candidiasis, and protecting *bg/bg-nu/nu* mice from lethal candidiasis than *B. infantis*. These results show that *Bifidobacterium* spp. can protect immunodeficient mice from candidiasis but different species manifest quantitative and qualitative differences in their probiotic and biotherapeutic effects.

Key words

Probiotics, *Bifidobacterium,* Candidiasis, Immunodeficient mice

Efectos bioterapéuticos de *Bifidobacterium* spp. en las candidiasis orogástrica y sistémica en ratones inmunodeficientes

Resumen

Se ha comparado la capacidad de dos *Bifidobacterium* spp. comerciales (*Bifidobacterium infantis* y *Bifidobacterium lactis*) para proteger a ratones inmunodeficientes *bg/bg-nu/nu* and *bg/bg-nu/+* de las candidiasis orogástrica y letal. Ambas especies de *Bifidobacterium* prolongaron la supervivencia de ratones *bg/bg-nu/nu* neonatos y adultos colonizados por *Candida albicans*. Las bifidobacterias afectaron a la producción de anticuerpos frente a *C. albicans*, inhibieron la candidiasis diseminada, suprimieron la pérdida de peso asociada con la infección por *C. albicans*, inhibieron el crecimiento de *C. albicans* en el tracto alimentario, la candidiasis sistémica endógena y redujeron la gravedad de la candidiasis gástrica en ambas cepas de ratones. *B. infantis* inhibió la candidiasis sistémica endógena mejor que *B. lactis*; sin embargo, *B. lactis* era significativamente más eficaz que *B. infantis* en la inhibición de la colonización del tracto alimentario por *C. albicans*, en la supresión de la candidiasis gástrica y en la protección de los ratones *bg/bg-nu/nu* de la candidiasis letal. Estos resultados muestran que las especies de *Bifidobacterium* pueden proteger a los ratones inmunodeficientes de la candidiasis, pero que las diferentes especies presentan diferencias cuantitativas y cualitativas en sus efectos probióticos y bioterapéuticos.

Probióticos, *Bifidobacterium*, Candidiasis, Ratones inmunodeficientes

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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 [4-7]. *Bifidobacterium* spp. are commensal intestinal bacteria that are used as probiotics, i.e., live bacteria that can be used as food supplements to provide beneficial effects to the host [8,9]. Commercial sources sell cultures of *Bifidobacterium* spp. for use as probiotics.

In humans, *Bifidobacterium* spp., alone or with other bacteria, have been able to decrease colonic inflammation in the elderly [10], prevent colonization by opportunistic pathogenic enterobacteria in antibiotic-treated radiotherapy patients [11], prevent antibiotic-associated diarrhea [12], and prevent rotavirus diarrhea in infants [13]. In rodent experiments, *Bifidobacterium* spp. protected mouse pups from rotavirus diarrhea [14], reduced the rate of gut translocation by enteropathogenic *Escherichia coli* [15], and enhanced mitogenic responses by immune cells [16,17].

A good deal of evidence supports the probiotic activity of *Bifidobacterium* spp.; however, it is clear that studies are needed to document the capacity of different probiotic bacteria to produce beneficial health benefits for a host. In this study, we assessed the probiotic effects of two *Bifidobacterium* spp. (*Bifidobacterium infantis* and *Bifidobacterium lactis*), obtained from two different commercial culture sources, for their capacity to protect immunodeficient *bg/bg-nu/nu* and *bg/bg-nu/+* mice from orogastric candidiasis and systemic candidiasis of endogenous origin.

MATERIALS AND METHODS

Microorganisms. Commercial starter cultures of probiotic bacteria were obtained from the following sources: *B. infantis* was a human isolate obtained from Rhone-Poulenc, Madison, Wis. The *Bifidobacterium* Bb-12 isolate was provided by Chr. Hansen's Laboratory, Inc., Milwaukee, Wis. Dr. Mario Marcon (Children's Hospital, Columbus, Ohio) has determined, by ribosomal RNA typing, that both isolates used in this study have close identity with *Bifidobacterium animalis* (personal communication). Contrary to the latter information, *Bifidobacterium* Bb-12 has been reclassified as *B. lactis* [18], and will be referred to by that name in this manuscript. 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-Dickinson Microbiology Systems) at 37°C. Microbiological identification was verified using the API 50CH biochemical identification system (BioMerieux-Vitek, USA) and cellular fatty acid chromatography (MIDI, Inc., USA).

Mice. C57BL/6 *bg/bg-nu/nu* and *bg/bg-nu/+* mice [19] were obtained from breeding stocks maintained at the University of Wisconsin Gnotobiote Laboratory, Madison, (http://www.biostat.wisc.edu/ gnotolab/gnotolab.html). Germfree (GF) male *bg/bg-nu/nu* and female *bg/bg-nu/+* mice were mated to obtain litters of approximately equal numbers of nude and heterozygous mice. 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 *Bifidobacterium* isolates by swabbing their oral and anal orifices with 1 ml (1 X 10^7 CFU/ml) of inoculum. Mice colonized with either *B. infantis* or *B. lactis* 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*. Swabs soaked with inoculum were rubbed on the os and anus of each mouse and additional inoculum was added to food pellets and water bottles (1 ml per food pellet and 10 ml per 250 ml bottle of water) to assure colonization. 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 *B. infantis* or *B. lactis*. All mice were given autoclave-sterilized food and water *ad libitum*, and autoclave-sterilized bedding. Bacterial and fungal cultures were carried out weekly to verify the microbial integrity of the experiment.

Although not conducted simultaneously, the *B. infantis* and *B. lactis* experiments overlapped in time. The need for separate isolators for each organism required individual experiments, which were all conducted under identical conditions.

Survival and growth of immunodeficient mice. Survival of mice born to GF or gnotobiotic mothers was assessed at 4 and 8 weeks of age. Survival of *B. infantis*- or *B. lactis*-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 GF control mice.

Gastrointestinal (GI) tract colonization. Quantitative cultures of fecal pellets obtained from the mice at 3 days and weekly after colonization demonstrated that the mice were colonized by the microorganisms. *B. infantis*, *B. lactis* and *C. albicans* colonization of the GI 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 bacteria 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. The number of *C. albicans* or bacteria in the internal organs are reported as CFU/g (dry weight) tissue.

Histological evaluation. The tongues, esophagi, stomachs, hard palates, rectums, and the major internal organs of the mice were fixed in 10% formaldehyde in pH 7.4 PBS. The fixed tissues were dissected, embedded in paraffin, sectioned onto slides for staining with hematoxylin and eosin, Gomori methenamine silver stain for fungi,

and for a Gram stain. Tissue samples from all sections of the alimentary tract and from the major internal organs were evaluated by a pathologist for evidence of infection, using the following criteria: Histopathology score (0 to 5) in infected tissues – (0) no infection evident; (1) 1-10 microorganisms (yeast and hyphae of *C. albicans*)/high power field (HPF, 400X); (2) 10-50 microorganisms/HPF; (3) 50-100 microorganisms/HPF; (4) confluent microorganisms/HPF; and (5) confluent microorganisms/HPF with hyphal penetration of viable tissues (yeast and hyphae of *C. albicans*).

Isotypic immunoglobulin responses to probiotics, C. albicans *or probiotics and* C. albicans. Serum immunoglobulin production by *bg/bg-nu/nu* or *bg/bg-nu/+* mice that were monoassociated with *B. infantis*, *B. lactis*, or *C. albicans* or diassociated with *C. albicans* and *B. infantis* or *B. lactis* was measured using radial immunodiffusion, as previously described [6,7].

Statistical analyses. Repeated measures Analysis of Variance (ANOVA) was used to test for differences in numbers of viable bacteria and *C. albicans* in the alimentary tract and internal organs of mice. The culture data were $\log_{10}$ transformed to better meet the assumptions of ANOVA. ANOVA with the rank sum test was used to evaluate the significance of differences in histopathology scores of *C. albicans*-monoassociated or *C. albicans* and *B. infantis*- or *B. lactis*-diassociated mice.

RESULTS

Bifidobacteria colonization. Quantitative cultures of bifidobacteria in feces from mice 3 days or 12 weeks after colonization were similar. Average numbers of *B. infantis* in feces from groups of 10 mice colonized 4 to 12 weeks were 9.9 ± 0.3 and 10.3 ± 0.3 $\log_{10}$ CFU/g (dry weight) in *bg/bg-nu/nu* and *bg/bg-nu/+* mice, respectively. The average numbers of *B. lactis* in *bg/bg-nu/nu* and *bg/bg-nu/+* mice, respectively were 9.8 ± 0.2 and 9.8 ± 0.1 $\log_{10}$ CFU/g (dry weight). These levels of colonization by bifidobacteria were the same for adult germfree mice and for mice born to females colonized with a *Bifidobacterium* spp. No significant differences were evident in the number of bifidobacteria present in the intestinal tract of mice ($p < 0.05$).

Populations of C. albicans *in the GI tract.* Compared with *C. albicans*-monoassociated mice, *bg/bg-nu/nu* and *bg/bg-nu/+* mice that were diassociated with *C. albicans* and *B. infantis* or *B. lactis* had significantly fewer *C. albicans* in their stomachs and intestines (Table 1). Data in Table 1 also show that *B. lactis* suppressed *C. albicans* numbers in the intestines of *bg/bg-nu/nu* mice better than *B. infantis*.

Probiotic inhibition of systemic candidiasis by B. infantis *or* B. lactis. Compared to dissemination in 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 *B. infantis* or *B. lactis* in the alimentary tract reduced the incidence of disseminated candidiasis in *bg/bg-nu/nu* mice (Table 2). Less systemic candidiasis of endogenous origin in *bg/bg-nu/+* mice was detected in *B. infantis* than in *B. lactis*-colonized mice (Table 2).

Table 2. Inhibition of systemic candidiasis by *B. infantis* or *B. lactis*.

	bg/bg-nu/nu mice		*bg/bg-nu/+* mice	
Microbial status	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:				
B. infantis[c]	14[d]	4.6 ± 0.6	12[d]	3.6 ± 0.2
B. lactis	38[d]	3.6 ± 0.1	22	3.4 ± 0.2

a: % incidence= % mice with disseminated candidiasis, n= 13 to 21 mice/group.
b: No. of *C. albicans*= Mean ± SEM log10 CFU *C. albicans*/g homogenized (spleen, liver, and kidney) tissue (dry weight).
c: Results for *B. infantis* have been reported previously [7].
d: Significantly inhibited dissemination of *C. albicans* compared with monoassociated mice, P<0.05.

Histological examination of tissues from probiotic-colonized mice. Gastric candidiasis, which increased in severity over an 8 week colonization study of *C. albicans*-monoassociated *bg/bg-nu/+* mice, was inhibited by *B. infantis* and *B. lactis* (Table 3). Although both *B. infantis* and *B. lactis* inhibited gastric candidiasis, *B. lactis* inhibited gastric candidiasis significantly better than *B. infantis* (Table 3).

Table 3. Histopathology of gastric candidiasis in mice colonized with *C. albicans* and *B. infantis* or *B. lactis*.

	Average histopathology severity score[a] (no. of mice)					
	bg/bg-nu/nu			*bg/bg-nu/+*		
Microbial status	2 wk	4 wk	8 wk	2 wk	4 wk	8 wk
C. albicans	3 (11)	3 (6)	*	2 (37)	3 (12)	3 (17)
C. albicans plus:						
B. infantis[b]		4 (3)	2 (6)		1 (5)[c]	2 (8)[c]
B. lactis		0 (7)[c]	0 (1)		0 (15)[c]	1 (8)[c]

*Mice died before time point.
a: Histopathology score = (0) no evidence of infection; (1) 1-10 microorganisms/high power field (HPF), (2) 10-50 microorganisms/HPF, (3) 50-100 microorganisms/HPF (yeast and hyphae of *C. albicans*), (4) confluent microorganisms/HPF (yeast and hyphae of *C. albicans*), (5) confluent microorganisms/HPF with penetration of viable tissues (yeast and hyphae of *C. albicans*).
b: Results for *B. infantis* have been reported previously [7].
c: Significantly less than *C. albicans*-monoassociated mice, P<0.05.

Survival of mice co-colonized with a Bifidobacterium *sp. and* C. albicans. Adult *bg/bg-nu/nu* mice diassociated with *C. albicans* and *B. infantis* survived significantly longer than *C. albicans*-monoassociated mice; however 100% of the adult *bg/bg-nu/nu* mice diassociated with *C. albicans* and *B. lactis* survived for 12

Table 1. *Bifidobacterium* spp. inhibit *C. albicans* in the gastrointestinal tracts of gnotobiotic mice.

	Number of viable *C. albicans*[a]									
	bg/bg-nu/nu mice					*bg/bg-nu/+* mice				
Microbial status	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:										
B. infantis[b]	6.4 ± 0.7[c]	7.8 ± 0.3[c]	8.4 ± 0.2[c]	7.3 ± 0.2[c]	8.3 ± 0.1[c]	6.7 ± 0.1[c]	6.6 ± 0.1[c]	7.2 ± 0.3[c]	6.5 ± 0.2[c]	8.3 ± 0.1
B. lactis	5.7 ± 0.7[c]	6.0 ± 0.5[cd]	6.9 ± 0.6[cd]	6.5 ± 0.3[cd]	7.3 ± 0.1[cd]	6.7 ± 0.1[c]	6.6 ± 0.1[c]	6.6 ± 0.3[c]	6.1 ± 0.5[c]	6.8 ± 0.3[cd]

a: Mean ± SEM log10 CFU/g (dry weight) of *C. albicans* isolated from intestinal contents at 4 to 12 weeks (cumulative data) after colonization, n = 13-21 mice/group.
b: Results for *B. infantis* have been reported previously [7].
c: Significantly fewer CFU than *C. albicans*-monoassociated mice, P<0.05.
d: Significantly fewer CFU of *C. albicans* with B. lactis than *B. infantis*, P<0.05.

Table 4. Protection of immunodeficient mice from lethal candidiasis by *B. infantis* or *B. lactis*.

	% Survival (no. mice/group)			
	bg/bg-nu/nu		*bg/bg-nu/+*	
Microbial status	4 wk % (n)	8-12 wk % (n)	4 wk % (n)	8-12 wk % (n)
Adult mice				
C. albicans	50 (14)	0 (7)	100 (24)	100 (24)
C. albicans plus:				
B. infantis[a]	95 (19)[b]	61 (18)[b]	100 (18)	93 (15)
B. lactis	100 (9)[b]	100 (6)[b]	100 (21)	100 (12)
Newborn mice				
C. albicans	0 (15)	0*	82 (13)	100 (11)
C. albicans plus:				
B. infantis[a]	50 (18)[b]	100 (6)[b]	100 (21)	100 (15)
B. lactis	100 (18)[b]	50 (6)[b]	100 (20)	100 (13)

a: Results for *B. infantis* have been reported previously.
b: Significantly increased survival compared to *C. albicans*-monoassociated control, p<0.05 by ANOVA and the Rank Sum test.

Table 5. Effects of *B. infantis* or *B. lactis* on *C. albicans*-induced body weight loss of adult mice.

	Mean ± SEM body weight[a] (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
B. infantis[b]	28.5 ± 1.6[b]	24.7 ± 0.7	33.2 ± 1.4	33.1 ± 0.3
B. lactis	29.5 ± 1.4	23.0 ± 0.3	32.5 ± 0.7	35.8 ± 0.4
C. albicans	18.4 ± 2.5[c]	15.2 ± 0.3[c]	31.1 ± 0.6[c]	29.9 ± 3.0
C. albicans plus:				
B. infantis[b]	17.7 ± 0.2[c]	18.6 ± 1.1[cd]	33.9 ± 1.1[d]	35.[6] ± 0.[6d]
B. lactis	24.4 ± 2.1[c]	23.1 ± 0.5[cd]	35.7 ± 0.6[d]	30.9 ± 0.7

a: Mice were colonized 4 to 8 weeks with *C. albicans*. There were 3 to 11 mice/group.
b: Results for *B. infantis* have been reported previously [6,7].
c: Significantly lower body weight than germfree control, P<0.05.
d: Significantly greater body weight than the *C. albicans*-monoassociated mice, P<0.05.

Table 6. *B. infantis* or *B. lactis* protect neonatal mice from *C. albicans*-induced loss in body weight.

Microbial	Mouse	Body wt. at 4 wk. age		Body wt. at 8 wk. age	
status	genotype	Male	Female	Male	Female
Germfree	*bg/bg-nu/nu*	18.8 ± 2.2[a]	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
B. infantis[b]	*bg/bg-nu/nu*	18.9 ± 0.6	12.0 ± 0.4	18.5 ± 3.4	21.5 ± 0.8
	bg/bg-nu/+	22.6 ± 0.6	18.0 ± 0.5	28.8 ± 0.2	23.7 ± 0.3
B. lactis	*bg/bg-nu/nu*	15.9 ± 1.9	13.7 ± 0.5	24.7 ± 0.4	21.4 ± 0.7
	bg/bg-nu/+	24.7 ± 0.1	21.5 ± 1.3	30.2 ± 0.7	25.0 ± 0.6
C. albicans	*bg/bg-nu/+*	7.1 ± 0.6[c]	21.7 ± 2.9	11.7 ± 1.1[c]	19.4 ± 0.5[c]
C. albicans plus:					
B. infantis[b]	*bg/bg-nu/+*	9.6 ± 0.3[cd]	24.6 ± 1.5	16.9 ± 2.2[cd]	23.8 ± 1.0[d]
B. lactis	*bg/bg-nu/+*	21.1 ± 1.4[d]	17.2 ± 0.7[c]	26.2 ± 0.5[cd]	21.7 ± 0.03[cd]

a: Experimental results, n = 3 to 14 mice/group.
b: Results for *B. infantis* have been reported previously [6,7].
c: Significantly lower body weight than germfree control, P<0.05.
d: Significantly greater body weight than the *C. albicans*-monoassociated mice, P<0.05.

weeks after oral challenge with *C. albicans* (Table 4). Compared with *C. albicans*-monoassociated mice, neonatal *bg/bg-nu/nu* mice survival was significantly prolonged in mice diassociated with *C. albicans* and either *B. infantis* or *B. lactis* (Table 4). We observed 50% mortality (before 4 weeks of age) of neonatal *bg/bg-nu/nu* mice diassociated with *C. albicans* and *B. infantis*; however, no further mortality occurred between 4 and 12 weeks. In contrast, neonatal *bg/bg-nu/nu* mice diassociated with *C. albicans* and *B. lactis* exhibited no mortality up to 4 weeks of age, but 50% mortality occurred between 4 and 12 weeks of age (Table 4). Neonatal and adult *bg/bg-nu/+* mice survived colonization with probiotic bacteria alone, with *C. albicans* alone or when diassociated with a probiotic bacteria and *C. albicans* (Table 4).

Effects of B. infantis *or B. lactis and* C. albicans *on adult body weights. C. albicans*-monoassociated male and female *bg/bg-nu/nu* and male *bg/bg-nu/+* mice had significantly smaller body weights than adult GF mice (Table 5). Monoassociation with either *B. infantis* or *B. lactis* did not decrease the body weights of mice as much as *C. albicans* (Table 5). Adult *bg/bg-nu/nu* mice diassociated with *C. albicans* and either *B. infantis* or *B. lactis* were significantly smaller than GF controls, but female mice in the latter groups were significantly larger than *C. albicans*-monoassociated controls (Table 5).

Effects of B. infantis or B. lactis *and* C. albicans *on growth of neonatal mice.* Body weights of *B. infantis*- or *B. lactis*-monoassociated *bg/bg-nu/nu* or *bg/bg-nu/+* mice, at 4 and 8 weeks of age, were not significantly different from those of GF mice (Table 6). Most *bg/bg-nu/+* mice born to *C. albicans*-monoassociated dams were significantly smaller than their age-matched GF counterparts (Table 6). Mice diassociated with *B. infantis* and *C. albicans* or *B. lactis* and *C. albicans* had better growth rates than *C. albicans*-monoassociated mice (Table 6).

Immune responses. Mice (*bg/bg-nu/+* and *bg/bg-nu/nu*) monoassociated with *B. infantis*, but not *B. lactis* had increased serum IgG, IgA, and IgM compared with sera from GF controls (Table 7). Serum IgG and IgA was significantly increased in *bg/bg-nu/+* mice, but not *bg/bg-nu/nu* mice after monoassociation with *C. albicans* (Table 7). In comparison to GF control sera, the levels of IgG, IgA, and IgM were elevated in *bg/bg-nu/nu* and *bg/bg-nu/+* mice diassociated with *C. albicans* and *B. infantis* (Table 7). The *bg/bg-nu/nu* mice that were diassociated with *C. albicans* and *B. lactis* produced IgG, but no IgA or IgM was detected (Table 7). The *bg/bg-nu/+* mice that were diassociated with *C. albicans* and *B. lactis* had increased serum IgG, IgA, and IgM in comparison to GF controls (Table 7).

Table 7. Immunoglobulins in sera of gnotobiotic mice monoassociated with *Bifidobacterium* spp. or *C. albicans* or diassociated with a *Bifidobacterium* spp. and *C. albicans*.

	Mouse	Immunoglobulin in mouse sera (µg/ml)[a]		
Microbial status	genotype	IgG	IgA	IgM
Germfree	*bg/bg-nu/nu*	293 ± 51	< 200	28 ± 2
	bg/bg-nu/+	301 ± 123	< 200	26 ± 9
B. infantis[b]	*bg/bg-nu/nu*	2431 ± 1651	299 ± 99	399 ± 255[c]
	bg/bg-nu/+	1792 ± 830	407 ± 56[c]	281 ± 95[c]
B. lactis	*bg/bg-nu/nu*	457 ± 95	<200	93 ± 11[c]
	bg/bg-nu/+	710 ± 161	234 ± 19	32 ± 7
C. albicans	*bg/bg-nu/nu*	1936 ± 1049	229 ± 29	32 ± 7
	bg/bg-nu/+	2257 ± 121	894 ± 21[c]	54 ± 12
C. albicans plus:				
B. infantis[b]	*bg/bg-nu/nu*	2179 ± 367[c]	1106 ± 39[c]	108 ± 26[c]
	bg/bg-nu/+	3269 ± 418[cd]	1212 ± 52[c]	155 ± 27[c]
B. lactis	*bg/bg-nu/nu*	91 ± 19[c]	<200	<2.5[d]
	bg/bg-nu/+	1326 ± 497[c]	704 ± 82[c]	69 ± 12[c]

a: Mean ± SEM, 5 mice/group, colonized 4 to 8 weeks.
b: Results for *B. infantis* have been reported previously [6,7].
c: Significantly different from germfree mice, P < 0.05.
d: Significantly different from *C. albicans*-monoassociated mice, P < 0.05.
The limits of detection for IgA levels was 200 µg/ml.

DISCUSSION

As probiotic bacteria, *Bifidobacterium* spp. hold promise for providing benefits to the host that include: protection of neonatal animals from pathogenic viruses, bacteria, and fungi [6,7,15,20], production of growth promoting factors for the host [21], providing adjuvant activity for antigens of pathogenic bacteria [16], and functioning as anti-inflammatory agents [10]. In a recent study, we compared the capacity of *B. infantis* with *Lactobacillus acidophilus*, *Lactobacillus reuteri*, and *Lactobacillus casei* to protect immunodeficient *bg/bg-nu/nu* mice from lethal candidiasis, and we found *B. infantis* to be more protective than the *Lactobacillus* spp. [7]. In the present study, we compared the capacities of *B. infantis* and *B. lactis*, from two different culture sources, to protect mice from candidiasis. The two *Bifidobacterium* spp. promoted similar protective effects against candidiasis.

The two isolates, although from different sources, are both closely related, genetically, to *B. animalis* [22]. Recently, the *Bifidobacterium* Bb-12 isolate from Chr. Hansen's Laboratory was identified as a new species, *B. lactis* [18]. It is important to consider that the identification and nomenclature of *Bifidobacterium* spp. is currently undergoing changes, which creates confusion in the identification of isolates being used for probiotics.

Our previous research indicated that *B. infantis* colonized the alimentary tracts of *bg/bg-nu/nu* mice and prolonged their survival after colonization with *C. albicans*, in comparison to *C. albicans*-monoassociated controls [7]. In this study, both probiotic bifidobacteria prolonged the survival of *bg/bg-nu/nu* mice after colonization with *C. albicans* (compared to *C. albicans*-monoassociated mice).

An important attribute of probiotic bacteria is their capacity to reduce systemic infections of endogenous origin by enteric pathogens [23]. We previously reported that *B. infantis* inhibited the dissemination of *C. albicans* from the gut to internal organs in *bg/bg-nu/nu* and *bg/bg-nu/+* mice [7]. In this study, both *Bifidobacterium* isolates suppressed (compared with dissemination in *C. albicans*-monoassociated mice) the incidence of disseminated candidiasis; however, whereas *B. infantis* protected both *bg/bg-nu/nu* and *bg/bg-nu/+* mice, *B. lactis* significantly protected *bg/bg-nu/nu* mice, but not *bg/bg-nu/+* mice, from disseminated candidiasis.

In a previous study [7], *B. infantis* was unable to protect *bg/bg-nu/nu* mice from gastric candidiasis, compared to gastric candidiasis in *C. albicans*-monoassociated mice. In this study, *B. lactis* was able to protect *bg/bg-nu/nu* mice against gastric candidiasis. The latter results suggest that species of *Bifidobacterium* differ (perhaps by different mechanisms of probiotic protection) in their capacity to protect mice from gastric candidiasis.

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 [7]. In this study, prior colonization with either *B. infantis* or *B. lactis* resulted in lower numbers of *C. albicans* in their alimentary tracts than in *C. albicans*-monoassociated mice. There was a significantly greater suppression of *C. albicans* in the alimentary tracts of mice colonized with *B. lactis*, than with *B. infantis*. The latter results also support the observation that *Bifidobacterium* spp. differ in their capacity to inhibit *C. albicans* in the murine intestinal tract.

We previously 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 *C. albicans*-induced weight loss [7]. In this study, the bifidobacteria protected adult female *bg/bg-nu/nu* and *bg/bg-nu/+* mice from weight loss that occurs in *C. albicans*-colonized mice; however, the diassociated mice still weighed less than GF controls. The growth of newborn *bg/bg-nu/nu* and *bg/bg-nu/+* mice colonized with *C. albicans* (i.e., born to *C. albicans*-colonized mothers) is also suppressed, in comparison to the body weights of GF controls [7]. In this study, the growth of male and female *bg/bg-nu/+* pups born to *C. albicans* and *B. infantis*- or *C. albicans* and *B. lactis*-colonized mothers was significantly improved at 4 weeks of age compared to the growth of pups born to *C. albicans*-monoassociated mothers. Thus, both bifidobacteria were able to protect the pups against *C. albicans*-induced weight loss.

Except for a significantly better suppression of disseminated candidiasis by *B. infantis*, *B. lactis* was found to be more efficient at protecting mice (e.g., by inhibition of *C. albicans* colonization of the alimentary tract, suppression of gastric candidiasis, and protection of adult *bg/bg-nu/nu* mice from lethal candidiasis) than *B. infantis*. The better protection afforded by *B. lactis* than *B. infantis* may be related in some ways to host adaptation of the *B. lactis* strain in these mice. Some authors have reported that host adaptation (survival) is important for probiotic organisms to produce the therapeutic effects [9,24,25]. Our results support the latter hypothesis since both bifidobacteria survived in the alimentary tracts of these mice; however, it should be noted *B. infantis*, was also quite effective at protection of immunodeficient mice from candidiasis.

In this study, the amounts of IgA, IgM, and IgG in sera from the *Bifidobacterium* spp. and *C. albicans*-colonized mice were measured to ascertain whether the differences in protective capacities of *B. infantis* and *B. lactis* for mucosal and systemic candidiasis could be explained immunologically. Indeed, *B. infantis*-monoassociated *bg/bg-nu/nu* and *bg/bg-nu/+* mice had higher levels of serum immunoglobulins than *B. lactis*-monoassociated mice, and *bg/bg-nu/+* mice diassociated with *C. albicans* and *B. infantis* had more IgG production than the *B. lactis* and *C. albicans*-diassociated mice. This ability of *B. infantis* to induce antibody production better than *B. lactis* could explain why *B. infantis* protected the mice better against disseminated candidiasis than *B. lactis*.

In conclusion, two *Bifidobacterium* spp., obtained from two different commercial sources, were compared for their capacity to protect immunodeficient mice from orogastric and systemic candidiasis. Our data show that the isolates (identified as the same species, *B. animalis* by ribosomal RNA typing) manifested different probiotic properties against candidiasis *in vivo*. *B. infantis* provided better protection of immunodeficient mice from disseminated candidiasis, whereas *B. lactis* protected mice from gastric candidiasis better than *B. infantis*. Overall, our results show that different isolates of bifidobacteria can provide important protective effects against candidiasis in immunodeficient mice.

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