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Comparison of *Lactobacillus* Strains with Respect to Bile Salt Hydrolase Activity, Colonization of the Gastrointestinal Tract, and Growth Rate of the Murine Host

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The significance of bile salt hydrolase production by lactobacilli in the microecology of the murine intestinal tract has not been extensively studied previously. Assays of bile salt hydrolase (sodium taurocholate as substrate) associated with cell extracts of five *Lactobacillus* strains of murine origin gave a range of activities (from 915 nmol of cholate released per mg of protein per 30 min to none detected). All of the strains tested colonized the murine gastrointestinal tract equally well. The growth rates of mice were not affected by colonization of their intestinal tracts by lactobacilli whether or not the bacteria produced bile salt hydrolase.

Lactobacilli are detected in large numbers in all regions of the murine gastrointestinal tract (8) and are responsible for most of the bile salt hydrolase activity detected in intestinal contents (11). Bile salt hydrolases catalyze the hydrolysis of conjugated bile acids, which results in the production of a free amino acid (taurine or glycine) and an unconjugated bile acid molecule. The concentrations of unconjugated bile acids in the small bowels of mice colonized by lactobacilli are higher than those of animals that do not harbor a *Lactobacillus* population (13).

Few strains of gastrointestinal lactobacilli have been characterized in terms of bile salt hydrolase activity. The significance of this enzyme as a colonization factor for these bacteria is not known. Thus, we compared the bile salt hydrolase activities of cell extracts of five strains of lactobacilli of murine origin. Extracts were prepared from *Lactobacillus* MRS medium (Difco, Detroit, Mich.) cultures grown for 16 h (stationary growth phase) under anaerobic conditions. Bacterial cells, harvested by centrifugation, were washed once in ice-cold sterile water and then suspended in water to give a 1/10 volume of the original culture. Cells were disrupted with a precooled cell press (American Instrument Co., Silver Spring, Md.) at 1.38×10^5 kPa. The resulting preparation was centrifuged at $17,400 \times g$ to remove cell debris, and the supernatant was filtered (0.45-μm-pore-size filters) and retained for the determination of bile salt hydrolase activity. The protein contents of preparations were measured by the method of Lowry et al. (4). Bile salt hydrolase activities in cell extracts were measured by determining the amount of [^{14}C]carboxyl-cholic acid released from [^{14}C]carbonyl sodium taurocholate (Amersham, Little Chalfont, United Kingdom) as described previously (11). Taurocholate was used as the substrate because activity against glycoconjugated bile salts was not detected when strains were tested by plate assay as described by Dashkevicz and Feighner (1).

A range of bile salt hydrolase activities was associated with the five strains examined (Table 1). Three strains had relatively high levels of activity (strains 100-14, 18, and 21), one strain had moderate activity (strain 20), and one strain lacked activity (100-93). The production of bile salt hydrolase was constitutive in all strains tested by comparing bile salt hydrolase activities in cell extracts prepared from cultures with and without 0.4 mM sodium taurocholate added to the medium as described by Lundeen and Savage (5).

To determine whether the production of bile salt hydrolase by a *Lactobacillus* strain influenced its ability to colonize the murine gastrointestinal tract, we inoculated groups of lactobacillus-free (LF) animals with three of these *Lactobacillus* strains (one strain per group of mice). The strains chosen for use in these experiments differed in the amounts of bile salt hydrolase they produced: strains 100-14 and 21 had high levels of activity, while in strain 100-93 there was an absence of activity. The mice used in these experiments were reconstituted lactobacillus-free (RLF) BALB/c mice from our colony and were derived and maintained in isolators by gnotobiotic technology as described by Tannock et al. (10). The animals harbored a gastrointestinal microflora functionally equivalent, on the basis of 26 microflora-associated characteristics, to that of conventional mice, but lactobacilli were absent. Mice were inoculated with the appropriate *Lactobacillus* strain by contaminating food pellets, fur, and bedding with MRS broth cultures of bacteria. Two weeks after inoculation, lactobacilli in gastrointestinal organs were enumerated by homogenizing forestomach, duodenal, jejunal, ileal, and cecal samples collected from mice after they had been killed by carbon dioxide anesthesia and cervical dislocation. Samples were homogenized in sterile saline to give a 10-fold dilution (wt/vol). Homogenates were further diluted and cultured on medium 10 agar plates selective for lactobacilli (6); they were incubated anaerobically for 48 h.

The numbers of lactobacilli detected in samples collected from various regions of the gastrointestinal tract were not statistically significantly different (Student's *t* test) no matter which strain had been used to colonize mice (Table 2). Therefore, we concluded that bile salt hydrolase was not an essential colonization attribute for lactobacilli, even in the proximal small bowel where the concentration of conjugated bile salts is highest because of the entry of bile into the intestinal tract via the bile duct.

In theory, deconjugation of bile acids in the small bowel by lactobacilli could be detrimental to the murine host since unconjugated bile acids are less efficient in the emulsification of dietary lipid and the formation of micelles than are conjugated molecules. Lipid digestion and absorption of fatty acids and monoglycerides could therefore be impaired by colonization of

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TABLE 1. Bile salt hydrolase activities of cell extracts of lactobacilli

Strain	Bile salt hydrolase activities[a]
Lactobacillus sp. strain 100-14	775, 1,054
L. delbrueckii 21	394, 466
L. delbrueckii 18	253, 271
L. fermentum 20	20, 27
Lactobacillus sp. strain 100-93	0, 0[b]

[a] Nanomoles of cholic acid released per milligram of protein per 30 min. Results of two assays are given.
[b] Release of radioactive cholic acid was no greater than that of the negative (cell extract not added) control.

the digestive tract by lactobacilli with bile salt hydrolase activity. Animals colonized by bile salt-hydrolyzing lactobacilli might thus gain weight more slowly than LF mice or animals that harbor only lactobacilli that lack bile salt hydrolase (2, 3). We tested the effect of colonization of the murine gastrointestinal tract by lactobacilli on the growth rates of mice with two strains of lactobacilli, strains 21 (high level of bile salt hydrolase activity) and 100-93 (absence of activity). These experiments utilized another colony of LF mice. LF mice were maintained in the same manner as the RLF colony, but they did not harbor streptococci, enterococci, lactobacilli, and some (undetermined) obligately anaerobic microbes as members of their digestive tract microflora (9). They were used in these experiments because they had very low levels of bile salt hydrolase activity in their intestinal tracts compared with those of conventional and RLF mice (11). In these experiments, the weights of animals that were the progeny of three breeding pairs of LF mice housed in the same isolator were determined at 3-day intervals between the ages of 21 (weaning) and 35 days. The births of litters to three breeding pairs were synchronized to occur on about the same day by a breeding management program, and each litter was reduced on the day of birth to five progeny per breeding pair. The weights of mice from these three litters were averaged for each day on which data were obtained. Preliminary experiments showed a linear relationship between age and weight for LF mice between the ages of 21 and 33 days. The rate at which weight was gained (growth rate) could thus be calculated from the slope of the curve when the results were subjected to regression analysis. Female mice gained weight at more consistent rates than did male mice so only data from the former were considered. The average growth rate of the first litter of LF mice was greater (around 0.52) than those of subsequent litters, whose rates were similar (around 0.48). Thus, data from the second and third litters were used to compare the effect of *Lactobacillus* colonization on murine growth rate. Three experiments were carried out. In the first experiment, the first two litters were maintained as LF mice. Before the birth of a third litter to each breeding pair, adult mice were inoculated with a culture of *L. delbrueckii* 21. In the second experiment, with different breeding pairs, adults were inoculated with strain 100-93 prior to the birth of the second litter and with strain 21 prior to the birth of the third litter. In the third experiment, with different breeding pairs, adults were inoculated with strain 21 prior to the birth of the second litter. The influence of bile salt hydrolase production by lactobacilli on the growth rates of mice could thus be determined for animals from a small genetic pool of inbred mice, raised in litters of identical size and maintained under identical conditions. LF mice colonized with lactobacilli had similar *Lactobacillus* populations, regardless of the strain involved (Table 2). Strain 21 produced bile salt hydrolase in vivo, as evidenced by significantly ($P < 0.05$) higher enzyme activity (equivalent to that of conventional mice [11]) in jejunal contents from mice colonized by this strain (mean [for 10 mice] nanomoles of cholic acid released per 30 min per gram [wet weight] of contents, 2,478, with a standard error of 746) than from LF mice (mean, 187 nmol, with a standard error of 23 nmol) and LF animals colonized by strain 100-93 (mean, 242 nmol, with a standard error of 50 nmol). No differences in growth rate were observed among LF mice, mice colonized by strain 100-93, mice colonized by strains 100-93 and 21, and mice colonized by strain 21 alone, nor were weight gains statistically (Student's *t* test) significantly different (Table 3). Colonization of the murine gastrointestinal tract by bile salt hydrolase-producing lactobacilli did not result, therefore, in slower growth of animals. In our experiments, LF mice were fed a standard rodent diet. It is possible that under conditions imposed by a less nutritious diet, bile salt hydrolase activity might assume more importance for host well-being. The *Lactobacillus* population of the gastrointestinal tract, however, is generally decreased when rodents are subjected to dietary stress (7, 12).

It can thus be concluded that bile salt hydrolase production is not an essential attribute for *Lactobacillus* strains that colonize the murine gastrointestinal tract. Further, the growth rates of mice that consume a nutritionally balanced diet are not affected by the presence of lactobacilli, bile salt hydrolase producing or not, in the gastrointestinal tract.

TABLE 2. Populations of lactobacilli in gastrointestinal tracts of mice

Mouse group (*n*)	Log_{10} lactobacilli/g (wet wt) of organ (SE)				
	Forestomach[a]	Duodenum	Jejunum	Ileum	Cecum
RLF colonized by 100-14 (5)	8.5 (0.3)	6.9 (0.3)	7.3 (0.4)	7.3 (0.3)	7.4 (0.3)
RLF colonized by 21 (5)	8.8 (0.2)	7.1 (0.3)	8.2 (0.2)	8.2 (0.3)	8.2 (0.3)
RLF colonized by 100-93 (5)	8.9 (0.3)	7.1 (0.2)	7.8 (0.2)	7.8 (0.2)	8.4 (0.3)
LF colonized by 100-93 (5)	ND[b]	5.8 (0.2)	7.2 (0.2)	7.5 (0.3)	ND
LF colonized by 100-93 and 21 (5)	ND	5.9 (0.3)	7.7 (0.3)	8.3 (0.2)	ND
LF colonized by 21 (5)	ND	6.5 (0.3)	7.3 (0.6)	8.0 (0.1)	ND

[a] Region of stomach lined by nonsecretory mucosae.
[b] ND, not determined.

TABLE 3. Mean growth rates and weight gains of female LF mice

Expt	Animal group	No. of mice	Growth rate[a]	Wt gain (g)[b]
1	LF	6	0.49	5.6 (0.2)
	LF colonized by 21	7	0.50	5.8 (0.1)
2	LF colonized by 100-93	10	0.48	5.5 (0.2)
	LF colonized by 100-93 and 21	6	0.48	5.6 (0.1)
3	LF colonized by 21	9	0.48	5.6 (0.1)
	LF colonized by 21	6	0.47	5.5 (0.2)

[a] Slope of regression curve obtained when mean weight was plotted against age.
[b] Mean weight gained from day 21 to day 33. Values in parentheses are standard errors.

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