Probiotics and Metabolism

The Association of Yogurt Starters with *Lactobacillus casei* DN 114.001 in Fermented Milk Alters the Composition and Metabolism of Intestinal Microflora in Germ-Free Rats and in Human Flora–Associated Rats[1]

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ABSTRACT   The aim of this study was to compare the effects of milk and of various fermented milks on the composition and metabolic activities of the intestinal microflora. Groups of eight rats were fed for 6 wk a diet containing 30% nonfermented milk (M), yogurt (Y), milk fermented with *Lactobacillus casei* (LcFM) or milk fermented with the association of *L. casei* DN 114.001 and yogurt starters (LcYFM). In the first study, the survival of the lactic acid bacteria from the fermented milks was assessed by bacterial enumeration in feces of germ-free rats (GF rats) fed milk or fermented milks. The metabolic activities of the lactic acid bacteria were studied in these rats by the measurement of glycolytic activities and products of bacterial fermentation, i.e., acetate and lactate (isoforms L and D). In a second study, the effects of fermented milks on the composition and metabolism [gas, glycolytic activities, short-chain fatty acids (SCFA), alcohol and ammonia] of human flora were studied using human flora–associated rats (HF rats). In GF rats, the survival of *L. casei* in the feces did not differ between those fed the LcFM and LcYFM diets. *L. bulgaricus* was detected in the feces of the rats fed Y, whereas *Streptoccus thermophilus* was found in the feces of the LcYFM group. In HF rats, fecal concentration of Bifidobacteria was greater in the LcFM group than in the others. β-Glucuronidase (EC 3.2.1.31) activity was lower in rats fed LcFM and Y than in those fed M and LcYFM, whereas β-galactosidase (3.2.1.23), α-glucosidase (EC 3.2.1 20) and β-glucosidase (EC 3.2.1.21) activities were higher in the LcYFM group compared with the others. Methane excretion was higher in rats fed Y than in other groups. Cecal SCFA concentrations did not differ in LcFM, Y and M groups, but total SCFA, acetate, propionate and butyrate were significantly greater in the LcYFM group. These results suggest that milk fermented with the combination of *L. casei* and yogurt starters leads to specific effects that are different from the simple addition of the effects found with yogurt and milk fermented with *L. casei*. These specific effects are potentially beneficial to human health.   J. Nutr. 127: 2260–2266, 1997.

KEY WORDS: • *fermented milk* • *gnotobiotic rats* • *human intestinal microflora* • *Lactobacillus casei*
• *yogurt*

The intestinal microflora is a complex ecosystem composed of a large variety of bacteria. The metabolic capacity of the flora is extremely diverse and can produce both positive and negative effects on the gut physiology (Gorbach 1986, Macfarlane and Cummings 1991). There is therefore great interest in the possibility of altering the intestinal microbiota in a beneficial way with the goal of improving the health of the host. Lactic acid bacteria have been considered potentially useful in this respect (Sanders 1993).

Lactic acid bacteria have been used traditionally in food fermentation for thousands of years. In fermented milks, these bacteria produce lactic acid as the primary end-product, along with acetic acid and ethanol, in the case of heterofermentative species. They also produce acetaldehyde, peptoglycan, pep-

tides, vitamins and antimicrobial substances that contribute to the taste, texture, and potential health benefits of the products (Hartley and Denariaz 1993). Yogurt remains a staple food and is fermented using a combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Other lactic acid bacteria are often combined with yogurt starters to produce fermented milks with specific desirable characteristics related to flavor or health properties (Robinson 1991).

Upon consumption, fermented milks deliver a large number of lactic acid bacteria into the gastrointestinal tract. These transiting microorganisms are capable of partially resisting gastric and bile acids and can therefore deliver enzymes and other substances into the intestines (Marteau and Rambaud 1993). Yogurt's ability to provide β-galactosidase and to decrease lactose intolerance symptoms in lactose maldigesters has been frequently reported (Marteau and Rambaud 1993, Sanders 1993).

Lactic acid bacteria have also been reputed to modify the intestinal milieu. Some studies have shown that *L. acidophilus*,

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L. casei and *Bifidobacterium bifidum* can modify potentially harmful bacterial activities such as those of β-glucuronidase and nitroreductase (Goldin and Gorbach 1984, Marteau et al. 1990). Few reports exist, however, that investigate the ability of lactic acid bacteria to alter the main fermentative processes of endogenous microflora. One recent study in humans showed that yogurt with or without *B. longum* did not modify fecal short-chain fatty acid (SCFA)[3] concentrations (Bartram et al. 1994).

An in vitro model simulating the various compartments of the gastrointestinal tract recently demonstrated that the survival rate of lactic acid bacteria in the gut varied, depending on whether the bacteria were associated with other bacteria or were tested alone (Havenaar et al. 1994). Thus, the ability of the lactic acid bacteria in fermented milks to have an effect on the intestinal tract in vivo may depend on their combination in the product.

The objective of this study was to compare the influence of the consumption of milk fermented with *L. casei* and yogurt starters (LcYFM) to those of yogurt (Y), milk fermented with only *L. casei* (LcFM) and nonfermented milk (M) on the composition and metabolic activities of the intestinal microflora. Two experiments were performed successively. In the first study, germ-free (GF) rats were used to evaluate both the survival of ingested bacteria through the gastrointestinal tract and their metabolic profile in vivo. In the second study, rats born germ-free and inoculated with human intestinal microflora (HF rats) were used to determine the effects of the ingested fermented milks on the composition and metabolism of the intestinal microbiota.

MATERIALS AND METHODS

Animals. Sixty-four GF male Fischer rats, 2.5 mo old (265 ± 7 g) were used. The rats, which originated from UEPSD breeding unit (INRA, Jouy en Josas, France), were reared in sterile Texler-type isolators (La Cahlène, Vélizy, France) filled with a rapid transfer system, in an environmentally controlled room (25°C) with a 12-h light:dark cycle. Rats were given free access to their diets and to sterilized water, autoclaved for 20 min at 120°C. Thirty-two rats were maintained in the GF state, and 32 were inoculated with 1 mL of a 10^{-2} dilution of fresh human feces from a methane producer (HF rats). Fecal dilution was performed in an anaerobic cabinet ($N_2/H_2/CO_2$, 85:10:5). All procedures were conducted in accordance with the institute's guide for the care and use of laboratory animals.

Experimental design. For 1 wk before the experiments, rats were given a human-like diet with the following composition (g/kg): mashed potatoes, 460; fish meal, 230; cellulose, 50; corn oil, 40; lard, 100; sucrose, 100; cholesterol, 0.15; and mineral and vitamin mixture, 20 (Andrieux and Sacquet 1986). The diet was sterilized by gamma irradiation at 45 kGy in plastic vacuum bags. When experiments started, GF or HF rats were assigned to four groups of eight and were transferred into sterile isolators, one isolator for each group. Rats were then given free access to diet offered as a paste containing 70% of powdered human-like diet that contain 30% M or one of three fermented milks, Y, LcFM or LcYFM. Milk and fermented milks were obtained from Danone (CIRDC, Le Plessis-Robinson, France). The products were conditioned in sterile pots and sealed with a double cover. The pots were provided on a daily basis to the isolators through a lock sterilized by peracetic acid (100 g/L). Milk and fermented milks contained the following (g/kg): protein, 3.7 and fat, 3.3. The fermented milks contained 5.2 g/kg and milk 12.7 g/kg of carbohy-

TABLE 1

Concentration of lactic acid bacteria in fermented milks, diets and in feces of germ-free rats fed the fermented milks diets[1,2]

	Y	LcFM	LcYFM
	log_{10} CFU/g		
Fermented milk[3]			
L. casei	0	8.7 ± 0.1	8.7 ± 0.1
L. bulgaricus	6.7 ± 0.2	0	6.6 ± 0.1
S. thermophilus	8.5 ± 0.1	0	9.1 ± 0.1
Diet[4]			
L. casei	0	8.2	8.2
L. bulgaricus	6.2	0	6.1
S. thermophilus	7.8	0	8.6
Feces[5]			
L. casei	0	9.3 ± 0.1	9.4 ± 0.2
L. bulgaricus	8.2 ± 0.2	0	ND
S. thermophilus	ND	0	9.0 ± 0.9

[1] Fermented milks: yogurt (Y), milk fermented by *L. casei* (LcFM) or milk fermented by yogurt and *L. casei* (LcYFM); CFU, colony-forming-units; ND: not detected (detection limit = 6 log_{10} CFU/g).

[2] Diets containing 30% fermented milks (Y, LcFM or LcYFM).

[3] Values are means $\pm$ SD ($n = 4$);

[4] Values estimated from the enumeration of lactic acid bacteria in the fermented milks.

[5] Values are means $\pm$ SD ($n = 8$).

drates. The lactic acid bacteria composition of fermented milks is listed in **Table 1**.

Collection of samples. In GF and HF rat studies, diets were given for a 4-wk period before fresh fecal samples (weight 0.1–0.2 g) were collected from each rat and immediately introduced into an anaerobic chamber to enumerate bacterial populations. Only HF rats were then transferred, for 4 d, into individual respiratory chambers, which could be connected and deconnected to the isolators to measure total hydrogen and methane production as previously described (LeCoz et al. 1989). After a 6-wk period, both GF and HF rats were killed by ether anesthesia, and their ceca were removed and weighed. Cecal pH was measured, and the contents were immediately frozen in liquid N_2 and stored at $-80°C$ until analyzed for enzymatic activities and bacterial metabolites.

Analyses. Lactic acid bacteria were analyzed in triplicate; three fecal samples from each GF rat were diluted from 10^{-6} to 10^{-9} in LCY medium[4] and then plated (0.1 mL) on selective agars. For *L. bulgaricus*, MRS (Man Rogosa and Sharpe) medium was incubated anaerobically at 44°C for 72 h. MRS with bactoxgall was used for *L. casei*, and M17 medium was used for *S. thermophilus*; plates for both were incubated aerobically at 37°C for 72 h. After incubation, colonies were counted and observed microscopically. Fecal samples of HF rats were diluted to 10^{-9} in an anaerobic Freter's chamber in the prereduced liquid medium LCY. Dilutions of 10^{-7}, 10^{-8} and 10^{-9} were plated (0.1 mL) on the following selective agars: BHI (brain heart infusion) neomycin medium for *Bacteroides*, Beerens medium for *Bifidobacteria*, GAPTS1 with azide for *Enterococci*, and DCA (desoxycholate acid) medium for *enterobacteria*. Plates containing BHI

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[3] Abbreviations used: CFU, colony forming units; GF rats, germ-free rats; HF rats, human flora–associated rats; LcFM *Lactobacillus casei* fermented milk; LcYFM, milk fermented by *Lactobacillus casei* and yogurt starters (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*); M, milk; SCFA, short-chain fatty acids; Y, yogurt.

[4] Culture media. The various media had the following compositions (g/L): LCY medium: casein enzymic hydrolysate (U.S. Biochemical, Cleveland, OH), 2; yeast extract (Difco, Detroit, MI), 2; NaCl, 5 and KH_2PO_4, 1. Man Rogosa and Sharpe medium (MRS) (Difco): at medium pH adjusted to 5.4, 55; MRS medium with bacto-oxgall, 1 (Difco). M17 medium: at pH 7.2, polytone (BioMérieux, Paris, France), 5; Soyase (BioMérieux), 5; yeast extract (BioMérieux), 2.5; meat extract (BioMérieux), 5; glucose, 5; ascorbic acid, 0.5; phosphate glycerol, 19; magnesium sulfate, 7; H_2O, 0.25. BHI medium: brain heart infusion (Difco), 37; yeast extract (Difco), 5; haemin, 0.005; BHI plus neomycin sulphate, 0.2. Beerens medium (Beerens 1990). GAPTS1 medium: yeast extract (Difco), 10; peptone, 15.0; tryptone, 10.0; sucrose, 1.0; sodium azide, 3.0 (pH 7.5). DCA agar medium: peptone, 10.0; lactose (Sigma, L'Isle d'Abeau Chesnes, France), 10.0; sodium deoxycholate (Sigma), 5.0; NaCl, 5.0; Na_2HPO_4, 2.0; iron citrate, 1.0; sodium citrate, 1.0; neutral red, 0.03.

TABLE 2

Cecal weight, pH, and bacterial metabolites in the cecal contents of germ-free rats fed milk or fermented milks[1]

	Diet[2]			
	M	Y	LcFM	LcYFM
Cecal pH	6.60 ± 0.06b	6.31 ± 0.10a	6.25 ± 0.10a	6.30 ± 0.11a
Cecal weight, *g*	16 ± 1	15.7 ± 1.5	16.3 ± 0.9	14.3 ± 1.7
Acetate, *μmol/g*	1.3 ± 0.2a	1.3 ± 0.4a	3.7 ± 0.2b	5.2 ± 0.5c
Total lactate,[3] *μmol/g*	4.3 ± 0.1a	11.1 ± 0.4b	39.8 ± 7.6c	37 ± 10c
L-Lactate, %	100	15	100	100
D-Lactate, %	0	85	0	0

[1] Values are means ± SD, n = 8. abc Within each line, unlike letters indicate significant differences (*P* < 0.05).
[2] Diets contained 30% milk (M), yogurt (Y), milk fermented by L. casei (LcFM) or milk fermented by yogurt and L. casei (LcYFM).
[3] Total lactate: L- + D-lactate; lactate data have been transformed as square roots for statistical analyses; nontransformed data are presented in the table.

or Beerens medium were incubated at 37°C for 48 h in an anaerobic chamber, and those containing GAPTS1 and DCA were incubated aerobically at 37°C for 48 h. Cultures were prepared in triplicate. Colonies were counted and bacteria were observed microscopically.

After HF rats were maintained in a respiratory chamber for 4 d, gases were collected from the chamber atmosphere using a 20-mL capacity plastic syringe equipped with a three-way valve. Hydrogen and methane were immediatly measured using a Quintron apparatus (DP-Quintron Instruments, ABS, Saint Dié, France).

The glycolytic activities investigated were related to the metabolism of food (β-galactosidase EC 3.2.1.23 and α-glucosidase EC 3.2.1.20) or were related to the release and enterohepatic recirculation of toxic substances (β-glucosidase EC 3.2.1.21 and β-glucuronidase EC 3.2.1.31). Enzyme assays were performed in an anaerobic chamber in 2-mL Eppendorf tubes. Glycolytic activity was measured by the rate of release of *p*-nitrophenols from *p*-nitrophenylglucoside. The reaction mixture contained 0.1 mL of a 5 mmol/L substrate solution and 0.2 mL of a 1:20 (v/v) dilution of the cecal sample in 0.1 mol/L phosphate buffer at pH 6.4. Incubation was at 37°C, and paranitrophenol concentration was measured according to the optical absorbency at 400 nm after the addition of 1.6 mL of 0.25 mol/L sodium carbonate. Enzyme activity was expressed as micromoles of product hydrolyzed per minute per gram of cecal sample.

SCFA, alcohol and lactic acid were measured in the cecal contents as markers of glycolytic fermentation, and ammonia and iso-acids as markers of proteolytic fermentation. SCFA and alcohol were analyzed after water extraction of acidified samples by using a gas-liquid chromatograph (Nelson 1020, Perkin-Elmer, St Quentin en Yvelines, France) equipped with a flame-ionization detector and a wide-bore column (15 m × 0.52 mm) (FSCAP Nukol, Supelco, L'Isle d'Abeau Chesnes, France) impregnated with SP 1000. Carrier gas (He) flow-rate was 10 mL/min, inlet temperature 175°C, column termperature 100°C, detector temperature 280°C, hydrogen and compressed air flow-rate 40 mL/min; 2ethylbutytrate was used as an internal standard.

L- and D-Lactate were determined enzymatically (Boehringer-Mannheim, Meylan, France), and ammonia was determined using the Berthelot method as adapted by Dropsy and Boy (1965).

Statistical analysis. Results are expressed as mean values and their standard errors. Data obtained from the study of GF rats and those obtained from the study of HF rats were analyzed separately by using one-way ANOVA (PCSM Software, Deltasoft, Meylan, France). If data were found to have heterogeneous variance, analysis was performed on data trasformed as square roots. If ANOVA indicated significant diet effects, means were compared using the Newman-Keuls test (PCSM Software). Statistical significance was accepted at *P* < 0.05.

RESULTS

Germ-free rat study. At the end of the experiment, body weight of GF rats ranged from 320 to 355 g (average 330 g) and intake ranged from 110 to 120 g per group without significant difference among treatment groups.

Lactic acid bacteria enumeration. The concentration of *L. casei* was more than 8 $\log_{10}$ colony-forming units (CFU)/g in LcFM and in LcYFM diets (Table 1). The concentration of *L. bulgaricus* in Y and LcYFM diets was 2 log lower than that of *S. thermophilus*. High levels of *L. casei* were found in feces of rats fed LcFM and LcYFM diets (Table 1). *L. bulgaricus* was detected in the feces of the Y diet group, but not in the LcYFM group. In contrast, *S. thermophilus* was not detected in the feces of rats fed Y, but was found in the feces of rats fed the LcYFM diet.

Bacterial metabolism. Compared with the results obtained in rats fed the M diet, rats fed fermented milk diets had a significantly lower cecal pH, with no difference in cecal weight (**Table 2**). In GF rats fed the M diet, only low concentrations of acetate and L-lactate were found. Acetate concentration was 1.8 times higher in rats fed LcFM and three times higher in those fed LcYFM than in those fed M or Y diets. Cecal lactate concentration was higher in rats fed fermented milk diets than in those fed the M diet. It was also significantly higher in rats fed the LcFM and LcYFM diets than in those fed the Y diet. D- and L-Lactate were produced in rats fed Y, but only the L isoform was found in rats given the LcFM and LcYFM diets. In the M group, only α-glucosidase and β-galactosidase were detected. In the Y group, β-galactosidase activity was significantly higher than in the M group, and β-glucuronidase was detected (**Table 3**). In rats fed the LcFM diet, β-galactosidase activity was as low as in the group fed the M diet, β-glucuronidase activity was not different from rats fed the Y diet and β-glucosidase was detected. In the LcYFM group, β-galactosidase, α-glucosidase and β-glucuronidase activities did not differ from those in rats fed the Y diet, whereas β-glucosidase activity was not different from that in rats μ fed the LcFM diet.

Human flora–associated rat study. At the end of the experiment, the body weight of HF rats ranged from 315 to 348 g and intake ranged from 105 to 120 g per group without significant difference among treatments.

Bacterial examination of the microflora. HF rats fed the M diet had fecal flora characterized by a larger population of *Bacteroides* than *Bifidobacterium, Enterococcus* and *enterobacteria* (**Table 4**). Levels of these populations were not different in rats fed Y. Cecal concentration of *Bifidobacterium* was higher in the LcFM group than in others, and although there was no significant difference in *Bifidobacterium* in rats fed the LcYFM diet, 50% of rats in that group had *Bifidobacterium* cecal concentration higher than 8 $\log_{10}$ CFU/g.

Bacterial metabolism. Daily hydrogen excretion did not differ among groups (**Fig. 1**), but methane excretion was 40% higher in rats fed the Y diet than in the other groups. Differences in cecal metabolism are shown in **Table 5**. The pH was

TABLE 3

Bacterial glycolytic activities in the cecal content of germ-free rats fed milk or fermented milks[1]

	Diet[2]			
	M	Y	LcFM	LcYFM
	μmol/(min · g cecal contents)			
β-Galactosidase	0.01 ± 0.002a	0.10 ± 0.01b	0.01 ± 0.003a	0.10 ± 0.01b
α-Glucosidase	0.17 ± 0.02	0.17 ± 0.02	0.15 ± 0.02	0.15 ± 0.02
β-Glucosidase	ND	ND	0.06 ± 0.02	0.07 ± 0.02
β-Glucuronidase	ND	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.01

[1] Values are means ± SD, n = 8; ND = not detected.
[2] Diets contained 30% milk (M), yogurt (Y), milk fermented by L. casei (LcFM) or milk fermented by yogurt and L. casei (LcYFM).
ab Within each line, unlike letters indicate significant differences ($P < 0.05$).

significantly lower in rats fed the LcFM diet than in other groups. Only in the LcYFM group was the cecal SCFA concentration significantly higher than in the other groups. Acetate, propionate and butyrate were significantly higher in the LcYFM group than in others, but iso-acids (cumulated iso-butyrate and iso-valerate), L- and D-lactates, ethanol and ammonia concentrations were not different. When comparing the bacterial glycolytic activities (**Fig. 2**), β-galactosidase, α-glucosidase and β-glucosidase were significantly higher in the LcYFM group than in all other groups. β-Glucuronidase activity was significantly lower in rats fed the Y and LcFM diets than in those fed M or LcYFM.

DISCUSSION

One of our objectives was to determine if *L. casei* and yogurt starters alone or in association with each other in fermented milks are able to survive transit through the upper gastrointestinal tract. At the time of this study, methods did not exist for distinguishing between the lactic acid bacteria in fermented milks and the autochthonous intestinal microflora; thus, human flora–associated rats were not appropriate for measuring bacterial survival. We therefore used germ-free rats for this portion of our experiment because they afford an opportunity to study bacterial survival rate and bacterial interactions under in vivo conditions.

The results obtained demonstrate that survival of bacteria differs among bacterial species and does in fact depend on the combination of lactic acid bacteria in the product. *L. casei* survives transit through the gastrointestinal tract when consumed in fermented milk diets, both as a monoculture and in association with yogurt starters, as shown by the large number of viable cells found in the feces. This result is consistent with the fact that *L. casei* is both acid and bile resistant and remains viable at a pH range of 3.0–7.0 (Goldin et al. 1992). With the use of an in vitro model, Havenaar et al. (1994) demonstrated that different strains of *L. casei* were able to survive transit through the gastrointestinal tract and reach the ileum in quantities sufficient to exert a physiologic effect.

Survival of bacteria from yogurt differed depending on whether or not these organisms were associated with *L. casei*. When rats ingested the Y diet, *L. bulgaricus* was recovered in the feces, whereas *S. thermophilus* was not detected. Conversely, *L. bulgaricus* was not detected, but *S. thermophilus* was recovered when rats were fed the LcYFM diet. Other studies on the survival of yogurt cultures using germ-free mice have led to inconsistent results. Bianchi Salvadori et al. (1984) found that *L. bulgaricus* was capable of implanting in the stomach and the large intestine. On the other hand, Besnier et al. (1984) reported that a single gavage in rats resulted in rapid
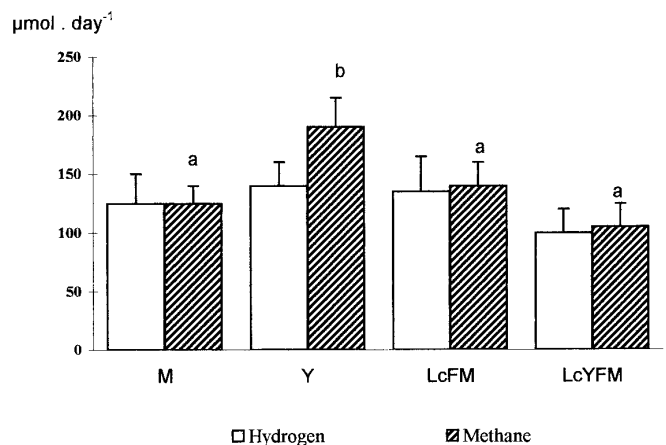
TABLE 4

Composition of the fecal microflora in human flora-associated rats fed milk or fermented milks diets[1]

	Diet[2]			
	M	Y	LcFM	LcYFM
	log₁₀ CFU/g			
Fecal microbiota				
Bacteroides	9.6 ± 0.1	9.8 ± 0.8	10.1 ± 0.9	10.1 ± 0.7
Bifidobacteria	7.4 ± 0.4a	7.8 ± 0.5a	8.5 ± 0.2b	8.2 ± 1.1a
Enterococci	7.9 ± 0.2	7.8 ± 0.8	8.0 ± 1.1	8.1 ± 0.9
Enterobacteria	6.8 ± 0.1	6.8 ± 0.4	6.8 ± 0.8	6.9 ± 0.6

[1] Values are means of 8 rats ± SD.
[2] Diets contained 30% milk (M), yogurt (Y), milk fermented by L. casei (LcFM) or milk fermented by yogurt and L. casei (LcYFM); CFU, colony-forming units.
ab Within each line, unlike letters indicate significant differences ($P < 0.05$).

FIGURE 1 Total daily production of hydrogen and methane in human flora–associated rats fed milk or fermented milks. Values are means ± SD, n = 8. Different letters indicate significantly different means ($P < 0.05$). Dietary group abbreviations: M, milk; Y, yogurt; LcFM, *L. casei* fermented milk; LcYFM, *L. casei* and yogurt starters fermented milk.

TABLE 5

pH and cecal concentrations of short-chain fatty acids (SCFA), L- and D-lactate, ethanol and ammonia in human flora–associated rats fed milk or fermented milks diets[1]

	Diet[2]			
	M	Y	LcFM	LcYFM
pH	6.3 ± 0.2 b[b]	6.3 ± 0.2[b]	5.9 ± 0.1[a]	6.1 ± 0.1[b]
	μmol/g			
Total SCFA	28.6 ± 1.0[a]	35.1 ± 2.4[a]	35.3 ± 3.5[a]	48.4 ± 3.4[b]
Acetate	17.0 ± 0.6[a]	21.7 ± 1.6[a]	21.3 ± 1.4[a]	30.4 ± 2.4[b]
Propionate	6.0 ± 0.6[a]	7.4 ± 0.4[a]	7.7 ± 0.4[a]	10.8 ± 0.8[b]
Butyrate	4.2 ± 0.3[a]	4.6 ± 0.4[a]	5.3 ± 1.0[ab]	6.1 ± 0.4[b]
Iso-acids[3]	1.4 ± 0.1	1.5 ± 0.3	1.0 ± 0.1	1.1 ± 0.1
L-Lactate	1.3 ± 0.1	1.1 ± 0.2	1.6 ± 0.2	1.1 ± 0.2
D-Lactate	5.9 ± 0.2	5.8 ± 0.2	5.8 ± 0.2	5.7 ± 0.5
Ethanol	1.0 ± 0.1	1.6 ± 0.4	1.5 ± 0.2	1.7 ± 0.4
Ammonia	5.8 ± 0.5	4.7 ± 0.3	4.9 ± 0.3	5.9 ± 0.6

[1] Values are means ± SD, n = 8; [ab] within each line, unlike letters indicate significant differences (P < 0.05).
[2] Diets contained 30% milk (M), yogurt (Y), milk fermented by L. casei (LcFM) or milk fermented by yogurt and L. casei (LcYFM).
[3] Iso-acids = iso-butyrate + iso-valerate.

fecal elimination of *L. bulgaricus*, whereas *S. thermophilus* remained at a high concentration in the feces. They further noted that the presence of both species persisted for 1 wk after discontinuation of the yogurt feeding. Interactions among bacterial species within the gut lumen may have led to reciprocal promotion of strains or to a partial or total inhibition of one or more species (Raibaud 1992).

The cecal contents of GF rats fed fermented milks correlates well with the metabolic profile of the lactic acid bacteria that survived in the colon: D-lactate was produced by *L. bulgaricus* in rats fed Y, and L-lactate was the major end product of *L. casei* and *S. thermophilus* in rats fed the LcFM or LcYFM diets. The slight production of acetate can most likely be attributed to *L. casei*, which was the only facultatively heterofermentative species used in the study (Hartley and Denariaz 1993).

Fermented milks likely improve the balance of the intestinal microflora either by supplying beneficial bacteria or by increasing the number and activity of endogenous bacteria that possess health-promoting properties. Such a hypothesis implies that ingested bacteria reach the colon in a live state. In humans, as we observed for GF rats, *L. casei* can be recovered in the feces during and for up to 2 wk after discontinuation of ingestion of the bacteria (Goldin et al. 1992, Ling et al. 1992, Saxelin et al. 1991). The ability of other lactic acid bacteria to survive is less clear. Bianchi Salvadori et al. (1978) found live *L. bulgaricus* and *S. thermophilus* in the feces of humans after yogurt consumption, whereas Pedrosa et al. (1995) did not. Hargrove and Alford (1978) found that in conventional rats, *L. bulgaricus* was not always present and *S. thermophilus* never survived beyond the upper small intestine.

When human microflora are inoculated in rats, they maintain their major metabolic characteristics (Andrieux et al. 1991). The specific changes observed in this study in the endogenous bacterial population and in bacterial metabolism of HF rats suggest that the lactic acid bacteria modified the intestinal medium in specific ways. We found that LcFM significantly increased the amount of endogenous *Bifidobacterium* in the feces. Similar effects have been described in humans (Hayatsu and Hayatsu 1993, Saxelin et al. 1991, Sepp et al. 1993).
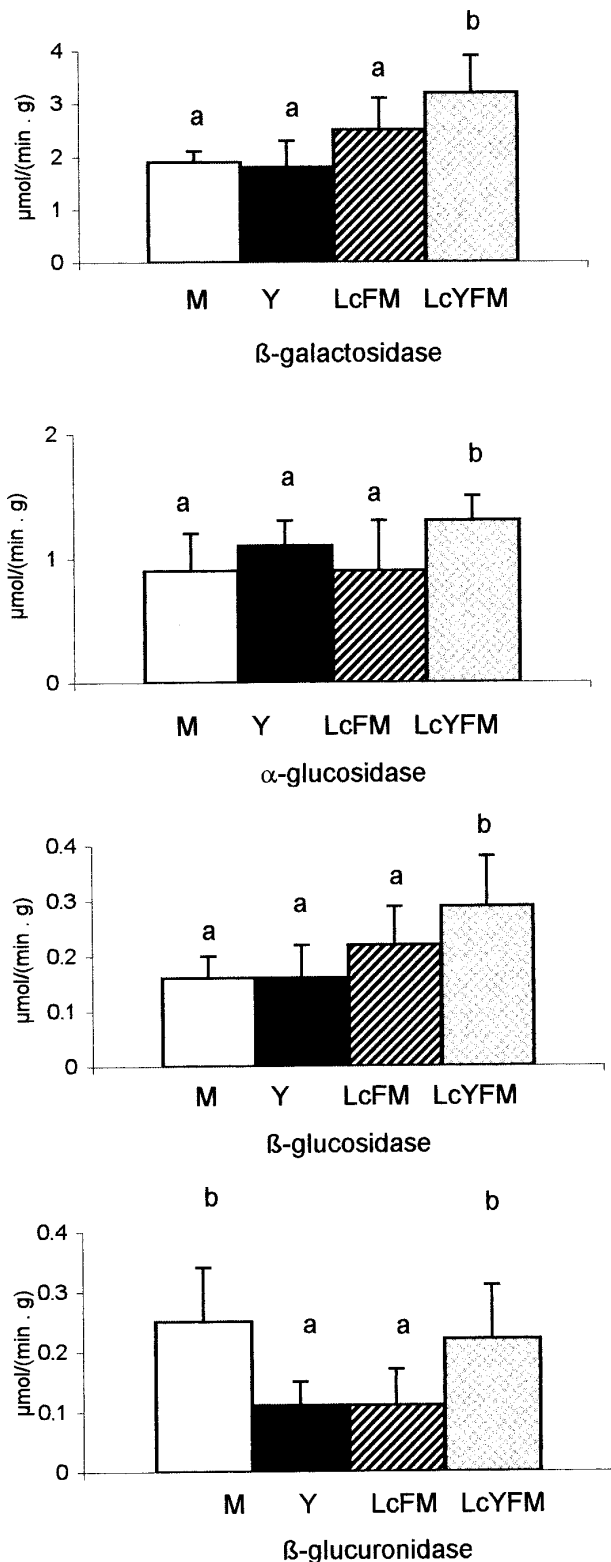

FIGURE 2 Bacterial glycolytic activities in cecal contents of human flora–associated rats fed milk or fermented milks. Values are means ± SD, n = 8. Different letters indicate significantly different means (P < 0.05). Dietary group abbreviations: M, milk; Y, yogurt; LcFM, *L. casei* fermented milk; LcYFM, *L. casei* and yogurt starters fermented milk.

This may be beneficial because *Bifidobacteria* have been associated with many health and nutritional benefits (Ballongue 1993). The low cecal pH observed in the LcFM group may have been due to the production of formic acid by *Bifidobact-*

eria, and not due to the survival of *L. casei* because lactate and acetate, the main products of *L. casei*, were not enhanced in cecal contents.

In addition to an increase in *Bifidobacterium*, LcFM significantly reduced β-glucuronidase activity, which has been implicated in colon carcinogenesis (Goldin and Gorbach 1984). Other reports have noted a decrease in β-glucuronidase activity in rats (Goldin and Gorbach 1984) and in humans (Ling et al. 1994) during ingestion of *L. casei*.

A decrease in β-glucuronidase activity may be related to a change in intestinal pH and/or to a modification in the composition of the intestinal flora. In our experiment, β-glucuronidase activity was lower in both LcFM and Y groups than in the M group; however, although the LcFM diet led to a decrease in pH and a change in *Bifidobacterium* population, the Y diet showed no influence on cecal pH or the composition of the endogenous intestinal microflora. *L. bulgaricus* was found in the feces of GF rats fed Y, and *L. casei* in those fed LcFM diet, and both bacteria had similar low levels of β-glucuronidase but different levels of β-galactosidase and fermentative metabolites, as shown in Table 3. These results suggest that lactic acid bacteria can potentially modify deleterious bacterial activity such as that of β-glucuronidase by more than one mechanism, depending on the species and strains present in the fermented milk. These mechanisms remain to be found.

We report here that the association of *L. casei* and yogurt starters in fermented milk (LcYFM) leads to a specific effect that clearly differs from the sum of the effects found in Y and LcFM. For LcYFM, neither pH nor β-glucuronidase activity was modified, and the amount of *Bifidobacterium* tended to be higher in only 50% of rats in this group. The particular effect of this new product was an increase in the activities of β-galactosidase and α- and β-glucosidase. In addition, there was a concomitant increase in total and specific SCFA concentrations.

These changes, specific to the LcYFM diet, may be attributable to the larger number of bacteria that reach the colon (twice that of Y and LcFM diets), as shown by the results in GF rats (Table 1). *L. casei* and *S. thermophilus* are both β-galactosidase producers and are able to hydrolyze carbohydrates other than lactose; they might provide glycolytic enzymes in the intestinal tract. Glycolytic activities, however, were low in GF rats compared with HF rats. Morevoer, the increase in acetate, propionate and butyrate in the ceca of HF rats fed the LcYFM diet without a concomitant change in lactate concentration is not characteristic of lactic acid bacterial metabolism. Thus, endogenous bacteria clearly are implicated in the glycolytic enzyme induction and fermentative process.

Whatever the mechanism for the above changes, the increase in acetate, propionate and butyrate may be beneficial for the host. These SCFA are major end-products of microbial fermentation. They are absorbed from the colon and are an important source of energy for tissues, and especially of butyrate, which is the preferred energy source for colonocytes (Macfarlane and Cummings 1991). In addition, the enhanced β-galactosidase and α-glucosidase activities found with the LcYFM diet offer a number of advantages for the host. The importance of β-galactosidase in improving lactose digestion is well recognized, and α-glucosidase may improve fermentation of resistant starch, which leads to butyrate production, improved bowel habits and increased stool output (Macfarlane and Cummings 1991). On the other hand, the increase in β-glucosidase activity is less clear because this activity has been implicated in both the generation of toxins (Mallett and Rowland, 1988) and of bacterial derivatives, which might be protective against chemically induced cancer (Roland et al. 1993).

In conclusion, our results point out differences in the survival of lactic acid bacteria in in vivo conditions, depending on the combination of bacteria in the fermented milk. They show that ingestion of lactic acid bacteria influences the composition and/or metabolism of endogenous microbiota. The new association of *L. casei* with yogurt cultures has specific effects of its own that differ from the simple addition of the effects of *L. casei*–fermented milk and yogurt. These effects may offer potential benefits for health.

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