Survival of Lactic Acid Bacteria in a Dynamic Model of the Stomach and Small Intestine: Validation and the Effects of Bile

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ABSTRACT

This study was conducted to validate a dynamic model of the stomach and small intestine to quantify the survival of lactic acid bacteria and to assess the influence of gastrointestinal secretions. The survival of a single strain of each of the following species, *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*, was measured under physiological conditions (e.g., peristalsis, changes in pH, and changes in concentrations of enzymes and bile) and were compared with data obtained from humans. No significant differences were found between the in vitro and in vivo data, indicating that the model has a predictive value for the survival of these bacteria in humans.

The survival of these strains of lactic acid bacteria in the gastrointestinal model was investigated under two different conditions in the small intestine: simulation of physiological secretion of bile and low bile secretion. Reductions in viability were significantly different between the bacterial species. The dose-response effect of bile on the survival of the tested bacteria was significant, demonstrating the bactericidal effect of bile salts. This study demonstrates the differences among bacterial species in their sensitivity to gastric and intestinal secretions.
(**Key words**: survival, lactic acid bacteria, gastrointestinal model, bile)

Abbreviation key: **GIT** = gastrointestinal tract.

INTRODUCTION

The survival of ingested microorganisms in the gastrointestinal tract (**GIT**) influences the risk of foodborne infections and the efficacy of probiotics and orally dosed live vaccines. Validated methods and models are required to study the mechanisms influencing the survival of microorganisms and to allow comparison and selection of probiotic or vaccinal strains. Ingested microorganisms are exposed during their transit through the GIT to successive stress factors that influence the survival of those microorganisms (20, 30). The roles of gastric pH and gastrointestinal peristalsis in preventing bacterial colonization of the small bowel are well established (12, 30); in contrast, the role of bile in this respect still is a matter of debate (30). Based on the results obtained in static in vitro models, some researchers (5, 30) have reported that the bactericidal effects of conjugated bile acids are weaker than those of free bile acids. However, this conclusion was questioned by others (31). In fact, the predictive value of results obtained in static in vitro models is limited for several reasons. First, the bile salt concentration in the gut is not static, but changes over time and in the different parts of the small intestine. After a meal, bile salt concentration sharply increases in the duodenum up to ca. 15 mmol/L and then progressively decreases to 5 mmol/L. In the jejunum, the bile salt concentration is ca. 10 mmol/L, and, in the ileum, the concentration falls below 4 mmol/L because of active ileal absorption (13, 24). Second, bile salts form micelles with phospholipids (as they are found in whole bile) and, therefore, have lower antibacterial activity than artificial solutions of pure bile salts (32). Finally, in vivo, the successive stresses by gastric acid and bile can be expected to exert a stronger antimicrobial effect than either of these parameters alone.

Recently, a dynamic, computer-controlled model has been developed that allows the simulation of successive in vivo conditions in the stomach and small intestine, such as the kinetics of pH, bile salt concentrations, and transit of the chyme (23). The objective of the present study is to validate this model in relation to the survival of ingested lactic acid bacteria and to assess the influence of gastric and biliary

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secretions on the survival of these bacteria. The species and strains used in this study were chosen because they are used in commercial fermented milks (6, 20) and because the survival of these bacteria has previously been quantified in humans (17, 19, 26, 27, 29), which allows validation of the model by comparison of the in vitro and in vivo results.

MATERIALS AND METHODS

Dynamic Gastrointestinal Model

Minekus et al. (23) have described the model: it comprises four serial compartments simulating the stomach, duodenum, jejunum, and ileum, which are connected by computer-controlled valve pumps (Figure 1). Temperature is kept at 37°C. The chyme is gently mixed three or nine times per minute in the gastric or intestinal compartment, respectively, by alternate contractions of the flexible walls. Simulated salivary, gastric, biliary, and pancreatic secretions are introduced into the corresponding compartments by computer-controlled pumps. The jejunal and ileal compartments are equipped with hollow fiber devices that permit dialysis of the chyme. The pH conditions in the gastric and duodenal compartments are monitored with pH meters connected to the computer. Secretion of either 1 mol of HCl or a neutral electrolyte solution into the gastric compartment is dosed via the computer for pH control. The same procedure is applied for secretion of either 1 mol of $NaHCO_3$ or the neutral electrolyte solution into the duodenal compartment. Mathematical modeling to reproduce and control gastric and intestinal emptying is performed using a power-exponential equation with variables for half-time of gastric or intestinal emptying and the β-value as a parameter describing the shape of the curve.

Products and Microorganisms

Two fermented milk products were used: Ofilus® (Yoplait, Paris, France), containing *Bifidobacterium bifidum* (ca. 10^8 cfu/g) and *Lactobacillus acidophilus* (ca. 10^7 cfu/g), and a yogurt containing *Lactobacillus delbrueckii* ssp. *bulgaricus* strain LB9 (ca. 10^7 cfu/g) and *Streptococcus thermophilus* strain ST20 (ca. 10^8 cfu/g). Ofilus® was studied 4 to 8 d after preparation as in the in vivo study described by Marteau et al. (19). Yogurt was prepared from one batch of milk powder (147 g/L). The reconstituted milk was sterilized at 110°C for 12 min, cooled at room temperature (± 20°C), and inoculated with *L. delbrueckii* ssp. *bulgaricus* strain LB9 and *S. thermophilus* strain ST20 (both kindly provided by Boll, Saint-Germain-les-Arpajon, France). The product was incubated aerobically at 37°C until the pH reached 4.6 (ca. 4 h) and was subsequently stored overnight at 4°C.

Experimental Design

The survival of the four bacterial species was assessed 1) inside the gastric compartment, 2) in the chyme delivered from the gastric compartment (gastric delivery), 3) in the chyme delivered from the ileal compartment during experiments simulating physiological bile salt concentrations, and 4) in the chyme delivered from the ileal compartment during experiments with low bile concentrations in the small intestinal model. For each condition, six experiments were performed. Two species were tested simultaneously: the two species in the Ofilus® product and the two species in the yogurt product. Gastric and ileal delivery experiments lasted for 3 and 6 h, respectively.

Before each experiment, the model was decontaminated by steaming at 100°C for 45 min. The Ofilus® and yogurt products (50 ml each) were separately introduced via an inlet on the gastric compartment after dilution (1:1, vol/vol) in a sterile electrolyte solution containing 6.2 g/L of NaCl, 2.2 g/L of KCl, 0.22 g/L of $CaCl_2$, and 1.2 g/L of $NaHCO_3$ to simulate the in vivo dilution by saliva. The secretion into the gastric compartment comprised the electrolyte solution with 370 U/ml of pepsinogen (Sigma Chemical Co., St. Louis, MO) at a flow rate of 0.25 ml/min and 1 mol HCl or the electrolyte solution at a flow rate of 0.25 ml/min. The pH curve in the stomach was computer controlled (23) to reproduce the values found in humans after yogurt consumption (3): pH 5.0 at initiation, pH 4.1 at 20 min, pH 3.0 at 40 min, pH 2.1 at 60 min, and pH 1.8 at >80 min. In the small bowel compartments, pH was kept at 6.5 ± 0.5.

Gastric and ileal emptying in the model were regulated by computer via the pump valves to reproduce the gastric and ileal emptying of a nonabsorbable meal marker that was ingested with yogurt by human volunteers (21). For gastric emptying, the half-time was 70 min, and the β coefficient of the power exponential equation was 2. For ileal emptying, the half-time was 160 min, and the β coefficient was 1.6 (23).

Duodenal secretion contained 1 mol of $NaHCO_3$ or the electrolyte solution (0.25 ml/min), 7% Pancreatin® (Pancrex V, Paines & Birne, Greenford, England) in 0.3 mol of $NaHCO_3$ (0.25 ml/min), and bile (porcine bile extract, which is comparable with

human bile; Sigma Chemical Co.) at a concentration differing among the experiments (flow rate 0.5 ml/min).

The dialysis fluid contained 5 g/L of NaCl, 0.6 g/L of KCl, 0.25 g/L of $CaCl_2$, and bile extract (concentration depending on the intestinal compartment and the experiment) and had a flow rate through the hollow fibers of 10 ml/min.

The concentrations of bile salts in the experiments simulating the physiological conditions were the same as those described by Minekus et al. (23). Briefly, 12.5 ml of 4% bile solution was in the duodenal compartment initially, followed by secretion of 4% bile for the first 30 min and 2% bile for the remaining time; the jejunal dialysis fluid contained 1.55% bile, and ileal dialysis fluid was without bile. The concentrations of bile salts during the low bile condition were kept at 2 mmol/L over time in each intestinal compartment. This concentration was obtained by initially introducing 20 ml of a 0.8% bile solution into the duodenal compartment, followed by the secretion of 2% bile; the dialysis devices were not used.

Sampling and Microbiological Methods

To study the effect of gastric secretion on the survival of bacteria, samples from the gastric compartment were taken at 0, 20, 40, 70, 106, 127, and 180 min after feeding. To assess the delivery of viable bacteria from the gastric compartment into the duodenal compartment, the chyme was collected on ice immediately after the pyloric valve and fractionated in 30-min periods for 3 h (intestinal compartments were not used in these experiments). The delivery of viable bacteria from the ileal compartment was assessed in chyme collected on ice from after the ileocecal valve and fractionated in 60-min periods for 6 h. Previous experiments have shown that storage of the samples on ice for 2 h did not affect the colony counts.

The volume of each sample was measured. Serial decimal dilutions were plated onto validated selective media with a spiral plater (Spiral System Instruments, Bethesda, MD). The following agar media and culture conditions were used: Rogosa medium (Oxoid Ltd., Basingstoke, England) for *L. bulgaricus* and *L. acidophilus* with anaerobic incubation (BBL® GasPak™; Becton Dickinson, Cockeysville, MD) at 37°C for 48 h, M17 (Oxoid) for *S. thermophilus* with aerobic incubation at 37°C for 48 h, and Beerens medium (1) for *B. bifidum* with anaerobic incubation at 37°C for 72 h.

Calculations and Statistics

The survival of bacteria were expressed as percentage of the ingested total number of bacteria (means ± SE), which allows the comparison of different species and different conditions, regardless of differences in initially ingested numbers of bacteria. In the gas-

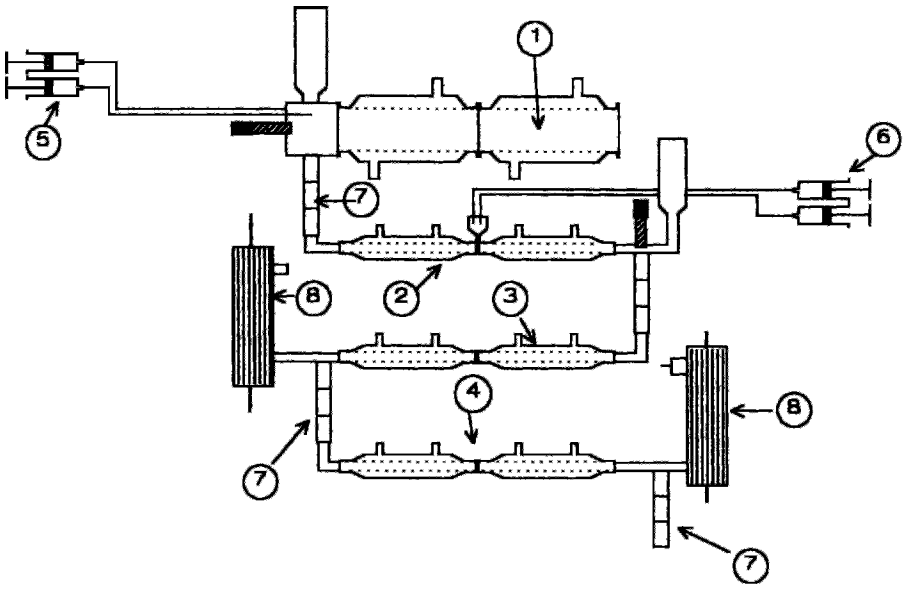

Figure 1. The dynamic multicompartmental model of the gastrointestinal tract: 1, gastric compartment; 2, duodenal compartment; 3, jejunal compartment; 4, ileal compartment; 5, gastric secretions; 6, intestinal secretions (bicarbonate, bile, and pancreas juice); 7, peristaltic valve pumps; and 8, dialysis devices connected to the jejunum and ileum.

tric compartment, the survival percentage was calculated in each sample, taking into account the volumes secreted and gastric emptying in time. Gastric and ileal deliveries of viable microorganisms were calculated from the bacterial counts in the samples and the corresponding outflow of chyme. The mean (± SE) initial viable numbers of lactic acid bacteria per milliliter in the gastric compartment of the model were $2.2 (\pm 1.2) \times 10^7$ and $3.6 (\pm 1.4) \times 10^6$ for *B. bifidum* and *L. acidophilus* (Ofilus® product), respectively, and $7.8 (\pm 1.4) \times 10^7$ and $2.1 (\pm 0.3) \times 10^8$ for *L. bulgaricus* and *S. thermophilus* (yogurt product), respectively. Cumulative percentages of the live bacteria delivered from the gastric and ileal compartments for the total collection period were obtained by summing the results of successive sampling periods.

The cumulative delivery of *L. acidophilus* and *B. bifidum* was compared with results obtained previously in human volunteers with the same bacterial species in the same product under similar conditions (19) using ANOVA. The in vitro and in vivo data were compared for bacterial survival in ileal samples that were taken within a 1-h interval in six replicates.

RESULTS

Streptococcus thermophilus and *L. bulgaricus* in the yogurt product survived only briefly in the gastric compartment (Figure 2). Viability after 40 min was significantly lower than that of *L. acidophilus* and *B. bifidum* in the Ofilus® product, and, within 70 and 110 min, the viable counts fell below 1% of the ingested numbers of bacteria. After 120 min, more than 40% of the ingested *L. acidophilus* and *B. bifidum* remained viable in the gastric compartment (Figure 2).

The deliveries of viable bacteria from the gastric compartment into the duodenal compartment (Figure 3) were significantly lower for *L. bulgaricus* (26%) and *S. thermophilus* (12%) than for *L. acidophilus* (64%) and *B. bifidum* (67%). The cumulative deliveries of viable *L. acidophilus* and *B. bifidum* cells from the gastric compartment increased continuously for more than 2 h (Figure 3), but those for *L. bulgaricus* and *S. thermophilus* reached a peak within 70 min.

The cumulative deliveries of the viable bacteria from the ileum into the colon, using the physiological and the low (2 mmol/L) bile concentrations, are shown in Figure 4. Passage through the small bowel with physiological bile concentrations resulted in a decreased survival of all four species relative to gastric delivery. At the low bile concentration, deliveries of viable *L. acidophilus* and *B. bifidum* were significantly higher. For *L. bulgaricus* and *S. thermophilus*, no differences were significant between physiological concentrations and low bile salt concentrations, probably because of the low survival (close to the detection limit) under both conditions.

The cumulative survival of *B. bifidum* and *L. acidophilus* during passage through the gastric and the small intestinal compartments (until the simulated ileo-cecal valve) did not differ significantly from

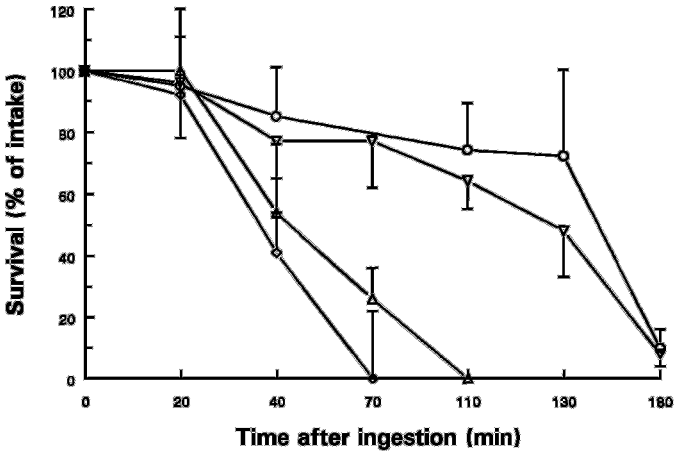

Figure 2. The survival of ingested *Bifidobacterium bifidum* (○), *Lactobacillus acidophilus* (▽), *Lactobacillus bulgaricus* (△), and *Streptococcus thermophilus* (◊) in the gastric compartment of the model. Values are expressed as mean percentages (±SE) of live bacteria relative to the ingested numbers (n = 6 for each strain).

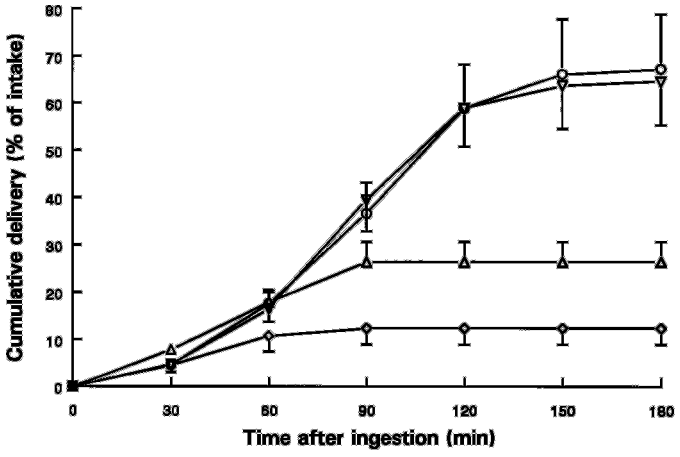

Figure 3. Cumulative delivery of *Bifidobacterium bifidum* (○), *Lactobacillus acidophilus* (▽), *Lactobacillus bulgaricus* (△), and *Streptococcus thermophilus* (◊) from the gastric into the duodenal compartment of the model. Values are expressed as mean percentages (±SE) of live bacteria passing the simulated pyloric sphincter relative to the ingested numbers (n = 6 for each condition).

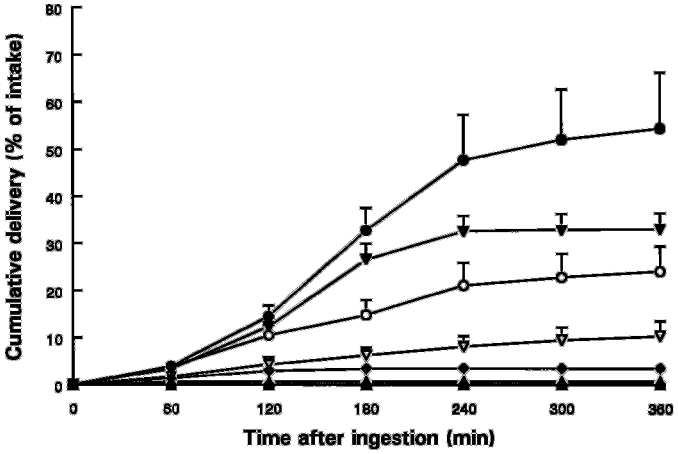

Figure 4. Cumulative delivery of *Bifidobacterium bifidum* (○,●), *Lactobacillus acidophilus* (▽,▼), *Lactobacillus bulgaricus* (△,▲), and *Streptococcus thermophilus* (◊,♦) from the ileal compartment, simulating physiological bile salt concentrations (open markers) and low bile salt concentrations (solid markers) in the small intestine compartments of the model. Values are expressed as mean percentages (±SE) of live bacteria passing the simulated ileo-cecal sphincter relative to the ingested numbers (n = 6 for each condition).

those found previously in humans with the same bacterial strains in the same vehicle (19) ($P = 0.88$).

DISCUSSION

Research on the survival of ingested bacteria in the GIT is important for the selection and development of probiotics or oral vaccines as well as for a better understanding of possible mechanisms underlying probiotic functions of beneficial microorganisms. Also, more knowledge is needed concerning the kinetics of survival of ingested pathogenic microorganisms in order to analyze the risk of foodborne pathogens (25). The survival of microorganisms has scarcely been quantified in vivo, except for some species of lactic acid bacteria (2, 17, 19, 26, 28, 29), because of difficulties in sampling from the human gut and because of ethical constraints. In some studies, attempts have been made to obtain information on the influence of acidic or bile salts on the survival of microorganisms using static single-compartmental models (3, 5, 8, 9, 14, 17, 28). However, the predictive value of such models is limited because they do not simulate the sequential stresses that are due to the continuously changing conditions to which ingested microorganisms are exposed during their passage in vivo. The model used in this study permits an accurate and dynamic simulation of the major factors influencing the survival of ingested microorganisms, such as pH, bile concentrations, and transit through the different parts of the GIT (23, 30).

Although the intakes of viable numbers of bacteria were standardized and were similar in each experiment, the expression of survival as a percentage of the total numbers of ingested bacteria facilitates comparisons among different species and under different conditions, and estimates the absolute amount of passing microorganisms. The data on survival of *L. acidophilus* and *B. bifidum* in the model are not significantly different from those obtained with the same strains in the same vehicle in healthy volunteers using an intubation technique (ANOVA: $P = 0.88$) (19). In addition, the cumulative numbers of viable bacteria passing the end of the intestinal compartments of the model were similar to those passing the ileum in humans (Figure 5). The data on survival of *L. bulgaricus* in the duodenal and ileal compartments (Figure 4) are also consistent with other data (17, 26, 27). Although the comparison with in vivo data is limited to one strain of four different species, this study shows the validity of the dynamic model and the method for the prediction of the survival of ingested lactic acid bacteria in humans.

The model can be used to assess the successive influences of gastric secretion, gastric emptying, and bile concentrations on the survival of probiotic microorganisms. Static experiments (3, 17) have shown that *Bifidobacterium* spp. and *L. acidophilus* are more acid-resistant than are *L. bulgaricus* and *S. thermophilus*. In this dynamic model, however, even for acid-sensitive species such as *L. bulgaricus* and *S.*

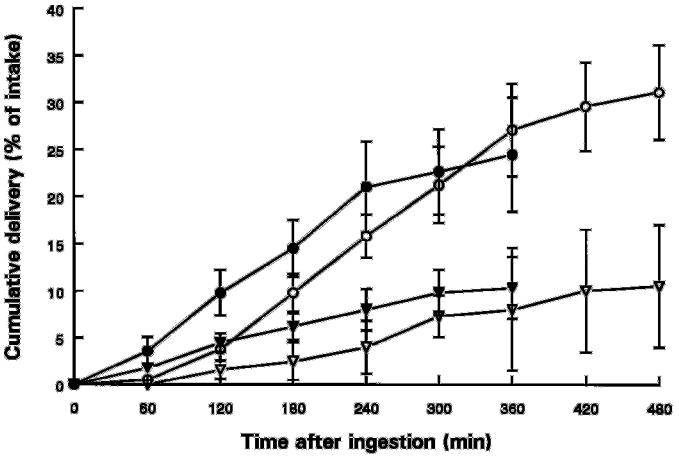

Figure 5. Cumulative delivery of *Bifidobacterium bifidum* (○,●) and *Lactobacillus acidophilus* (▽,▼) from the ileum of human volunteers (19) (open markers) and from the ileal compartment of the model (solid markers). Values are expressed as mean percentages (± SE) of live bacteria passing the simulated ileo-cecal sphincter relative to the ingested numbers (n = 6 for each condition).

thermophilus, a relatively large fraction of ingested bacteria reached the duodenum alive (Figure 3). This situation occurred mainly during the first 20 to 30 min after a meal when the pH in the stomach was still relatively high (above pH 3.8) (3, 27). This result emphasizes the importance of the initial period of gastric emptying for the delivery of live bacteria into the small intestine. The kinetics of gastric delivery simulating that of yogurt was relatively slow (half-time of gastric emptying was 70 min). Therefore, the survival of ingested bacteria would have probably been even higher if the fast gastric emptying of a liquid would have been simulated (with a half-time of gastric emptying of 30 min).

Until now, the lethality of bile on microorganisms in the human small bowel was thought to be low, even negligible (30), because of in vitro data that showed that conjugated bile salts, which constitute the majority of bile salts present in the small bowel, were less bactericidal than deconjugated bile salts (5, 13, 24, 30, 31). In the present study, bile exerted a strong influence on the survival of the bacterial species tested (Figure 4); the survival rate varied within a small range of bile concentrations. These findings support the importance of investigating the sensitivity of microorganisms to bile as a selection step for potential probiotics (6, 8, 11). The bile stress for ingested microorganisms in the GIT is complex because bile concentrations and residence times vary in each compartment of the GIT. Furthermore, the bile stress occurs after the pH stress in the stomach. Sublethally injured microorganisms have a different and unpredictable resistance to new stress factors (16). For these reasons, a dynamic model is expected to be more appropriate for prediction of the in vivo effects of bile on microorganisms than a static model with a constant concentration of bile.

Although probiotic microorganisms are generally thought to survive the transit through the GIT for their functionality (6, 20), the damaging effect of bile salts on yogurt bacteria also seemed to have positive consequences. Bile could liberate the lactase activity from yogurt bacteria in the small bowel, which could partially explain the better lactose digestion after digestion of yogurt by lactase-deficient subjects (7, 15, 18, 22). The bacterial lysis in the small intestine depends on bile salt and could thus be considered as a way to deliver specific biologically active components to the duodenum using ingested microorganisms. Clinical applications can be investigated in this in vitro model, for example, lipase activity to treat pancreatic insufficiency for cases in which classical lipase delivery systems are not sufficiently active in the duodenum (4, 10).

CONCLUSIONS

The described dynamic in vitro model of the gastrointestinal tract offers new possibilities for quantitative study of the survival of microorganisms in the gastrointestinal lumen. This model can be helpful in screening microorganisms for targeting in the gut, such as probiotics or oral vaccines, whether or not in combination with specific food components as selective substrate (prebiotics). Finally, the influence of certain microorganisms on the metabolic activity in the lumen can be studied.

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