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Survival, Physiology, and Lysis of *Lactococcus lactis* in the Digestive Tract

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The survival and the physiology of lactococcal cells in the different compartments of the digestive tracts of rats were studied in order to know better the fate of ingested lactic acid bacteria after oral administration. For this purpose, we used strains marked with reporter genes, the *luxA-luxB* gene of *Vibrio harveyi* and the *gfp* gene of *Aequora victoria*, that allowed us to differentiate the inoculated bacteria from food and the other intestinal bacteria. Luciferase was chosen to measure the metabolic activity of *Lactococcus lactis* in the digestive tract because it requires NADH, which is available only in metabolically active cells. The green fluorescent protein was used to assess the bacterial lysis independently of death. We report not only that specific factors affect the cell viability and integrity in some digestive tract compartments but also that the way bacteria are administrated has a dramatic impact. Lactococci which transit with the diet are quite resistant to gastric acidity (90 to 98% survival). In contrast, only 10 to 30% of bacteria survive in the duodenum. Viable cells are metabolically active in each compartment of the digestive tract, whereas most dead cells appear to be subject to rapid lysis. This property suggests that lactococci could be used as a vector to deliver specifically into the duodenum the proteins produced in the cytoplasm. This type of delivery vector would be particularly appropriate for targeting digestive enzymes such as lipase to treat pancreatic deficiencies.

Lactic acid bacteria are widely used in industrial food fermentation, contributing to the flavor, texture, and preservation of fermented products. These bacteria are generally regarded as safe, and certain strains can be used to treat human diseases (14). The improvement of cell engineering technology opens new possibilities, extending the potential for use of lactic acid bacteria as biotherapeutic agents from a few natural strains to recombinant strains (9). These strains might produce heterologous proteins such as enzymes (lipase and lactase), biological mediators (hormone and interleukin), and molecules stimulating local immune responses to prevent digestive pathologies (toxins and viral proteins) (9). Previous work has shown that genetically modified bacteria ingested orally were able to provoke a local immune response even when the antigen location was cytoplasmic (26, 31). It remains unknown how the oral inoculation of antigens expressed intracellularly can induce a specific immune response. A simple explanation could be that cytoplasmic content was liberated after bacterial lysis in the digestive tract (DT). In this case, the extent of the lysis and the area in which it occurs may be critical to obtain an optimal response.

The viability of lactic acid bacteria in the DT was studied for many different species, including the two yogurt bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (21, 24). The survival rate of these bacteria in the upper gastrointestinal tract appeared to be low (21). Pochart et al. observed that this survival rate was about 1% (24). However, it is still unknown if bacterial death is followed by a rapid lysis of the cells.

In order to investigate the fate of lactic acid bacteria in the DT, we studied the survival and the physiology of *Lactococcus lactis*, the model species for lactic acid bacteria, in the gastrointestinal tracts of rats. We used two well-established reporter gene systems. The first, the bacterial luciferase of *Vibrio harveyi*, was used already to investigate *L. lactis* gene expression and metabolic activities in the DT (10). The second, the green fluorescent protein (GFP) of *Aequora victoria*, allowed us to study the survival of bacteria in complex environments (3, 19, 33).

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MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The bacterial strains and plasmids are listed in Table 1. *L. lactis* strains were grown in M17 (29) with 0.5% glucose at 30°C. *Escherichia coli* was grown in Luria broth medium (Difco) at 37°C. Erythromycin was used at a concentration of 10 μg/ml. *Bacillus stearothermophilus* spores were used as a transit marker and germinated at 60°C in G-spore medium (12).

Isolation of plasmid DNA and enzyme analysis. Plasmid DNA was isolated by the method of Holmes and Quigley (17) for *E. coli* and by the method of Anderson and McKay (1) for *L. lactis. E. coli* was transformed by the heat shock method (27). *L. lactis* was transformed by electroporation of cells grown in the presence of glycine to weaken the cell wall (18). Restriction and modification enzymes were purchased from Boehringer and used according to the supplier's instructions.

Expression of GFP in *L. lactis*. The gene encoding GFP was amplified from the plasmid pGFPuv (Clontech) by PCR with the primers 5′-GAT*CCCCGGG***AAG GAGG**AGAAAAAATGAGT-3′ and 5′-CGGCGCTCAGTTG*GAATTC*ATTA-3′. In addition to restriction sites (italic letters), a new ribosome binding site (RBS) was designed (boldface letters). This fragment was inserted into the *Sma*I-*Eco*RI sites of pBluescript II KS(+) (Stratagene), forming the plasmid pJIM2924. This plasmid was digested by *Spe*I, blunted, and then inserted in the *Bam*HI site blunted of pJIM2289, a derivative of pJIM2279 (25) containing the deregulated promoter of the *L. lactis* histidine operon, P*hisD3* (11). The resulting plasmid, pJIM3001 carries the *gfp* gene under the control of P*hisD3*.

Animal model. (i) Force-feeding of *L. lactis*. Six female Fischer rats (6 to 8 weeks old) each received 0.5 ml of a *L. lactis* culture (approximately 10^9 CFU/ml of inoculum) mixed with *B. stearothermophilus* spores (approximately 4×10^8 CFU/ml of inoculum). A tube was passed through the mouth and into the stomach, allowing the direct injection of a known volume of bacterial suspension. The force-fed animals were slaughtered 1 h after inoculation.

(ii) Transit of *L. lactis* with diet. We wanted to study what happens to bacteria ingested with a conventional meal. The alimentation process of conventional rodents thus had to be modified, since rodents nibble all night. We adapted six conventional rats to receive a powdered diet mixed with one volume of M17, once per day, over a 2-h period. In this condition, the rats ate rapidly. On the day of the experiment, the M17 was replaced by a culture of *L. lactis* in M17 (10^9 or 10^{12} CFU/ml of food) mixed with *B. stearothermophilus* spores (approximately

TABLE 1. Bacterial strains and plasmids

Bacterial strain or plasmid	Description	Source or reference
E. coli		
TG1	*supE thiD*(*lac-proAB*) *hsdD5* F^+ *traD36 proAB lacIZ*ΔM15	15
L. lactis		
IL 1403	Plasmid-free	6
JIM4886	IL 1403 carrying pJIM2289	10
JIM5647	IL 1403 carrying pJIM3001	This work
Plasmid		
pGFPuv	Amp^r, carrying a derivative of the *gfp* gene of *A. victoria*	Clontech
pBluescript	Amp^r, *E. coli* cloning vector	Stratagene
pJIM2924	pBluescript carrying the *gfp* gene	This work
pJIM2279	Ery^r, cloning vector for gram-positive bacteria	25
pJIM2289	Ery^r, pJIM2366 containing P*hisD3* upstream of the *luxAB* gene	10
pJIM3001	Ery^r, derivative of pJIM2289 deleted from *luxAB* and carrying the *gfp* gene under the control of P*hisD3*	This work

4×10^8 CFU/ml of food). The animals were slaughtered 30 min after the food was removed.

Survival of *L. lactis* in the DT. The total contents of the stomach, small intestine (divided in three equal parts corresponding to duodenum, jejunum, and ileum), and cecum were immediately collected after slaughtering, weighed, and diluted (1/3) in sterile water. *L. lactis* JIM4886 or JIM5647 (carrying an erythromycin-resistant plasmid) and *B. stearothermophilus* counts were estimated from these dilutions for each sample. *L. lactis* survival was estimated on selective M17 agar plates containing erythromycin. In this condition, the autochthonous microflora did not interfere with the *L. lactis* enumeration. The spores were used as a transit marker to follow the inoculum in the bulk of the DT. They do not grow, are not destroyed in the DT, and are easily enumerated on agar plates at 60°C, where intestinal bacteria do not usually grow (12). The survival percentage of *L. lactis* in the different DT compartments was calculated with respect to the spore counts, as follows: [(count of *L. lactis* in one DT compartment/count of spores in the same compartment)/(count of *L. lactis* in the inoculum/count of spores in the inoculum)] × 100.

In vitro studies of *L. lactis* survival. The effect of the DT contents on *L. lactis* was tested in vitro by mixing washed *L. lactis* cells with DT extracts or enzymes. *L. lactis* cultures were centrifuged and resuspended in the same volume of water or tested extract and incubated for 30 min, 1 h, 1.5 h, or 2 h at 30°C. After incubation, cells were collected to be analyzed. The digestive extracts of stomach and duodenum from rats fed for 2 h with a powdered diet supplemented with M17 were sampled. These samples were centrifuged in order to clear the supernatant of debris and intestinal bacteria. Purified pepsin or trypsin was suspended in water at 5 mg/ml and used at a final concentration of 1 mg/ml in the bacterial cell suspension. Trypsin inhibitor (type II-S, soybean; Sigma, St. Louis, Mo.) was used at a final concentration of 4 mg/ml.

Assay of luciferase and GFP produced by *L. lactis*. Luciferase activities were measured immediately after the addition of 5 μl of nonaldehyde (Sigma) to samples diluted in water (25). Light emission was measured in a photometer (LB9501; Bertold). The results were expressed thereafter as micro-relative light units per CFU. This assay excludes from the measure dead cells that are unable to emit light.

The GFP activities were analyzed on cell samples fixed on a plate with paraformaldehyde in phosphate-buffered saline. To visualize all bacteria, including those native to the DT, samples were colored with 4,6-diamino-2-phenylindole (DAPI) (2.5 μg/ml; Sigma) added directly to the glycerol–phosphate-buffered saline mountant (Citifluor Ltd., Canterbury, United Kingdom) (2). Lactococci colored with DAPI can be detected with a 360-nm-excitation filter. Lactococci expressing the GFP can be specifically detected by epifluorescence with a 500-nm-excitation filter.

RESULTS

Influence of the method of administration on cell survival in the DT. In the first experiment rats were force-fed with a suspension of *L. lactis* JIM4886 and spores of *B. stearothermophilus* as a control for dilution. The *L. lactis* and *B. stearothermophilus* suspension was injected directly into the stomach with a syringe. The contents of the DT were collected 1 h after force-feeding. The viability of the bacteria was affected in each compartment of the DT (Table 2). The higher lethality was observed in the duodenum and jejunum, where only 0.01 and 0.4%, respectively, of the bacteria survived, while 6% survived in the stomach. The higher survival rates in the ileum and cecum (40 and 16%, respectively) were due to the fact that the injected bacteria rapidly reached these lower DT compart-

TABLE 2. Survival of *L. lactis* JIM4886 in the different compartments of the DT[a]

DT compartment	Survival counts for bacteria that were:								
	Force-fed			Mixed with diet					
				10^9 CFU/ml			10^{12} CFU/ml		
	No. of bacteria[b]	No. of spores[b]	% Survival[c]	No. of bacteria	No. of spores	% Survival	No. of bacteria	No. of spores	% Survival
None (inoculum)	1.0×10^9	4.8×10^8		1.0×10^9	4.8×10^8		1.0×10^{12}	4.8×10^8	
Stomach	6.0×10^6	4.0×10^7	6	6.4×10^8	2.8×10^8	91	2.7×10^{11}	1.2×10^8	90
Duodenum	7.9×10^3	2.5×10^7	0.01	4.7×10^7	6.3×10^7	30	3.2×10^7	1.2×10^7	0.1
Jejunum	4.0×10^5	4.0×10^7	0.4	2.8×10^8	2.6×10^8	43	4×10^8	1.0×10^8	0.2
Ileum	4×10^7	4.0×10^7	40	4.5×10^8	2.6×10^8	64	4×10^9	1.6×10^8	1
Cecum	7.9×10^6	2.0×10^7	16	5.0×10^8	2.0×10^8	100	3.2×10^{11}	2.0×10^8	64

[a] Bacteria were administered in suspension by force-feeding or mixed with the diet at 10^9 CFU/ml or at 10^{12} CFU/ml. Spores of *B. stearothermophilus* were mixed with *L. lactis* cells at 4×10^8 spores/ml.

[b] Viable count expressed in CFU per milliliter of inoculum or CFU per milliliter of DT content.

[c] The survival percentage of *L. lactis* was calculated with respect to the spore counts: [(count of *L. lactis* in one DT compartment/count of spores in the same compartment)/(count of *L. lactis* in the inoculum/count of spores in the inoculum)] × 100.

ments, without spending much time in the upper ones. This observation was confirmed by using a dye marker (dextran blue). We observed that 2/3 of the small intestine was completely colored only 10 min after injection of the cell suspension containing dextran blue. Thus, the bacteria which reached the lower DT compartments were not affected by the adverse environmental conditions encountered in the upper ones.

In a second experiment, rats received the bacteria mixed with powder diet in one meal per day. The *L. lactis* JIM4886 viability, in particular in the stomach (where the survival rate was 91% instead of 6% as observed in the preceding assay), was less affected when the bacteria was mixed with diet than when it was administered pure by force-feeding (Table 2). Moreover, the percent viability was still lower in the duodenum and jejunum than in the other compartments of the DT, but the difference was reduced, since 30% of the bacteria mixed with the food survived in these compartments (Table 2). The viability was higher in the lower part of the DT (ileum and cecum). This might be explained by the fact that some of the transiting bacteria reach these DT compartments rapidly, without staying in the previous compartment, which would affect their survival.

In the third experiment we tested the effect of increasing the dose of *L. lactis* in the powdered diet. The culture was concentrated 1,000-fold before it was mixed with the powder diet (10^{12} CFU/ml of food instead of 10^9 CFU/ml of food). The viability was dramatically affected in the three compartments of the small intestine as shown, for example, by the fact that only 0.1% of bacteria survived in the duodenum compared to the 30% observed previously (Table 2). However, the bacterial survival rate in the stomach and the cecum was good, as the count was similar to that obtained with a lower concentration of cells in food (Table 2). We verified in parallel that the step of centrifugation had no impact on the *L. lactis* viability in the rat DT.

Energetics of surviving *L. lactis* in the DT. To study the metabolic state of the ingested bacteria, we used an *L. lactis* strain, JIM4886, expressing the bacterial luciferase of *V. harveyi* under the control of a constitutive promoter (10, 25). The light emission requires as a cofactor reduced flavin mononucleotide that should be supplied by bacteria. The luciferase activity is thus directly dependent on cells. Only viable and energized cells contain reduced flavin mononucleotide (10, 30). The luciferase activities were measured in the DT contents of each of the rats fed with JIM4886 either by force-feeding (data not shown) or in transit with the food. The values obtained were calculated according to the viable-lactococcus counts in the different DT compartments and then expressed in micro-relative light units per CFU of *L. lactis* (Fig. 1). The amounts of luciferase activity per CFU were not significantly different in the inoculum and the different DT compartments. The viable cells are thus metabolically active in the DT.

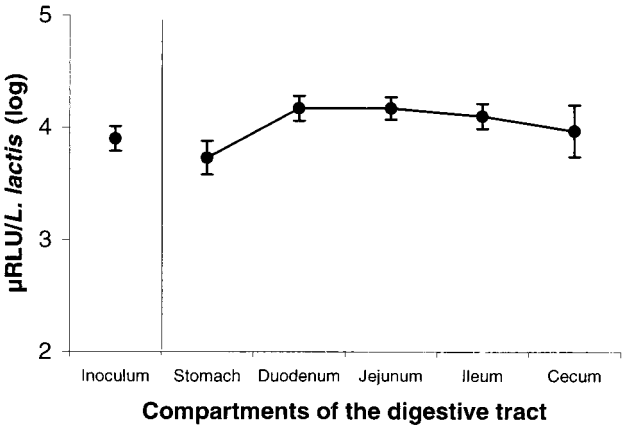

FIG. 1. Luciferase activity of *L. lactis* JIM4886 in the contents of the different compartments of the DT. RLU, relative light units.

Lysis of the nonenergized *L. lactis*. We have shown that viable cells were metabolically active, but the previous experiment did not allow us to determine if the dead cells were lysed or remained intact. A variant of the *A. victoria* GFP was used to estimate the fate of the nonviable cells in the DT. This protein glows bright green when exposed to ultraviolet light (4). Lactococci expressing GFP can be visualized after UV excitation as green fluorescent cocci. If *L. lactis* undergoes lysis in the DT, GFP is diluted in the medium and the bacteria are not fluorescent anymore. On the other hand, dead but intact cells will be visualized.

The number of bacteria (*L. lactis* JIM5647) surviving in the stomach and duodenal extracts were enumerated on selective plates and the results were expressed in CFU per milliliter of DT content. The results of this count, corrected by the dilution factor yielded by the spore enumeration are reported in Table 3. The percentage of surviving bacteria in the duodenum compared to that in the stomach is 0.2% when the bacteria are given concentrated in the food and 20% when the bacteria are not (Table 3). Fifty microscopic images of the same samples were analyzed with a fluorescence microscope with a DAPI filter and a GFP filter, and the results were expressed as CFU per 50 microscopic images. When the bacteria were given concentrated, the number of fluorescent cells was too high to be enumerated in the stomach samples while no fluorescent lactococci were detected in the duodenal samples. When the

TABLE 3. Counts of viable and intact lactococci in stomach and duodenal contents by enumeration of erythromycin-resistant *L. lactis* and of *L. lactis* expressing GFP by fluorescence microscopy

Culture	No. of viable cells[a] (mean ± SE)		No. of viable cells in duodenum/no. in stomach (%)	No. of intact cells[b] (mean ± SE)		No. of intact cells in duodenum/no. in stomach (%)
	Stomach	Duodenum		Stomach	Duodenum	
Concentrated	316,000 ± 17,000	630 ± 20	0.2	>500,000[c]	0[d]	<0.01
Not concentrated	620 ± 100	120 ± 40	20	1,680 ± 250	420 ± 20	25

[a] Viable count (10^6 CFU/ml of stomach or duodenum content) on M17 plus erythromycin, corrected by the dilution factor.
[b] Result of the count of GFP-positive fluorescent cells in 50 microscopic images, corrected by the dilution factor.
[c] Not countable with the standard dilution used in this experiment.
[d] No bacteria detected in 50 images.

TABLE 4. Counts of viable and intact *L. lactis* JIM4886 after 1 h of incubation in stomach or duodenal extract

Extract	Viable cell counts		Intact cell counts	
	No. of CFU[a] (mean ± SE)	Survival (%)	No. of cells[b] (mean ± SE)	Survival (%)
None (inoculum)	1,200 ± 120	100	1,400 ± 110	100
Stomach	1,170 ± 200	97	1,200 ± 160	86
Duodenum	40 ± 5	3	130 ± 30	9

[a] Viable count (10^6 CFU/ml of inoculum, stomach content, or duodenum content) on M17-plus-erythromycin plates.
[b] Results of enumeration of dead but intact cells visible in 50 images taken by microscopy with a DAPI filter.

bacteria were not concentrated in the food, 1,680 ± 25 and 420 ± 20 (means ± standard errors) fluorescent lactococci were found in the images corresponding to the stomach and duodenum samples, respectively. Because the ratio of the number of viable cells in the duodenum versus that in the stomach was similar to the ratio of intact cells (Table 3), the number of dead but intact cells is not significant in the duodenum. Taken together, these results strongly suggest that dead bacteria are rapidly lysing in the duodenum.

In vitro study of the DT factor affecting the survival of *L. lactis*. To study more precisely the factors affecting cell viability and lysis in the different parts of the DT, in vitro studies were developed. The bacterial cultures were resuspended and incubated in the stomach or duodenal clear extracts for 30 min, 1 h, 1.5 h, or 2 h. The viability of JIM4886 estimated by enumeration on a selective plate remained higher than 90% in the stomach extracts while it decreased rapidly to 3% in the duodenal extracts, independently of the time of incubation. Fifty images of bacteria from the samples incubated for 1 h were analyzed by microscopy with a DAPI filter to look for intact dead cells. The results of the intact cell count shown in Table 4 indicate that 86% of the bacteria remained intact in the stomach extracts versus 9% in the duodenal extracts.

In experiments that were similar but carried out with a duodenal supernatant heated at 80°C for 5 min before resuspending the cells, the survival rate increased significantly (28 versus 3% without heating). This shows that the cell death is in part dependent on a thermolabile factor. Interestingly, the incubation of *L. lactis* in a solution of trypsin strongly affected its survival, since only 0.012 or 0.0012% of the *L. lactis* bacteria survived after 30 min of incubation. In contrast, the survival rate in a solution of pepsin, a stomach enzyme, was 80%. Lastly, incubation of the cells in a duodenal supernatant in the presence of a trypsin inhibitor increased the survival rate to 25%, a value similar to the one obtained with the heated extracts. Trypsin might thus be an important factor involved in cell death.

DISCUSSION

In this study we have examined the survival, the metabolism, and the lysis of lactococcal cells in the different compartments of the DT with different protocols of ingestion with the aim of better knowing the fate of ingested *L. lactis* cells. For this purpose we used strains marked with reporter genes that allowed us to differentiate the inoculated bacteria from food and intestinal flora. In a previous study, we showed that luciferase could be used to measure the metabolic activity of *L. lactis* in the DT (10). In addition to this bioluminescent marker, we have expressed the GFP protein from *A. victoria* to study the rate of lysis in this complex medium. Our results show not only that specific factors affect the cell viability and integrity in some DT compartments but also that the way bacteria are administered has a dramatic impact.

Effects of diet and inoculum on bacterial survival. The protocol of *L. lactis* ingestion greatly influences the bacterial survival rate in the DT. For example, uptake of pure *L. lactis* culture by force-feeding led to a massive bacterial lysis in each compartment of the DT. With another protocol that simulated human digestion, *L. lactis* mixed with food survived well in the stomach and its viability in the duodenum was significantly increased. Interestingly, there was a more than 100-fold difference in the survival rate, showing survival to be a function of the concentration of bacteria in food. In the duodenum, a significant fraction of the population (10 to 30%) survived when the ingested population was moderate. In contrast, a massive lethality occurred with a 1000-fold-higher starting population. This result demonstrates that food may have a important protective effect on the bacteria present in diet. However, this protector effect might be limited to a certain level of population for a reason that is not understood yet. This protective effect of food and the inoculum size effect might be of crucial importance in the use of probiotics. They could be determinants in the choice of the way the bacteria will be constructed and administered in order to deliver therapeutic molecules.

Factors affecting *L. lactis* survival in the DT. In the force-feeding experiment, a part of the cell suspension injected into the stomach reached the lower DT parts rapidly, without spending much time in the upper ones. This model allowed us to assess factors specific to each part of the DT that may affect cell integrity independently of the stresses encountered earlier during digestion. It is well known that environmental conditions vary greatly between the mouth and the colon. The first stress encountered by bacteria in the DT is due to acidic gastric secretions (21). It was proposed that these constitute a major defense mechanism against the majority of ingested microorganisms. The ability of lactic acid bacteria to survive in gastric juices varies according to the species (8, 21). This study shows that pure *L. lactis* cells are sensitive in the stomach but become relatively resistant when mixed with food. Since the protective effect of food is independent of the size of the bacterial population in the stomach, a simple explanation is that food has a buffering effect that protects bacteria against the acidity due to gastric secretions. Acidity might thus be the major agent for the bacterial mortality in the stomach.

The conditions encountered in the duodenum have a more dramatic influence on lactococcal viability, since at best only 10 to 30% of ingested bacteria survive in this compartment. Bacterial viability in this compartment is affected by enteric secretions such as mucus, lyzozyme, and phospholipase A2 (16, 23). In addition, both mouse and human Paneth cells express several peptides with antimicrobial activities, called defensins (13, 20), that make the cell membranes of microorganisms permeable (28, 32). The duodenum is also the compartment where bile salts and pancreatic juices are secreted. In vitro experiments have demonstrated the bactericidal effects of bile salts on several lactic acid bacteria species (22). However, to our knowledge no data clearly demonstrates the effects of the pancreatic secretions in vivo. Strikingly, the protective effect of food in the duodenum varies as a function of the size of the bacterial population. This does not support the hypothesis that food has a buffering or diluting effect that protects bacteria, as is the case in the stomach. Heat-treated duodenum extracts lose a part of their lethal effect on *L. lactis*, suggesting that the inhibition is partly due to an enzyme. Treating duodenal extracts with a trypsin inhibitor reduced their destructive effect to

the same level as heat treatment. In addition, pure trypsin affected *L. lactis* viability in vitro, which leads us to believe that trypsin may be an important factor affecting the viability of *L. lactis* in the duodenum.

***L. lactis* has a low potential to produce therapeutic molecule in the duodenum.** The metabolic activities of lactic acid bacteria in the DT have not been documented in the past. However, knowledge of the physiology of the bacteria in the DT is necessary to fully develop their potential as biotherapeutic agents, especially if the molecule is to be produced in situ. To trace metabolically active bacteria in vivo, light-emitting genes such as luciferase can be used, since their activity depends on cofactors provided by the cell (7, 10). In this study, the amounts of luciferase activity per CFU were similar in the inoculum and in each compartment of the DT independently of the method of administration, suggesting that viable cells are metabolically active all the way through the DT. However, the high cell mortality rate observed in the duodenum considerably reduces the potential of lactococci to produce significant amounts of proteins in this segment of the DT. Their use might thus be restricted to target molecules into the cecum, and to a lesser extent the ileum. Since lactococci do not colonize the DT, their action will last only for 1 day; thus, they will need to be ingested daily during the treatment.

***L. lactis* is a potential delivery vector to target molecules in the duodenum.** Digestive enzymes such as lipase or lactase must be liberated in the duodenum. Since lactococci do not appear to be able to produce significant amounts of these molecules in this compartment, another targeting strategy must be used. One such strategy could be to induce cell lysis, so that the *L. lactis* protein contents, including the molecules produced during the preparation of the inoculum, will be liberated in large amounts in the duodenum. Since lactococci are not viable in the duodenum, we tested whether they also lysed spontaneously and thus could be used to directly deliver their content. To study the fate of the nonviable cells, we used a new luminescent reporter gene which encodes a derivative of the GFP from the jellyfish *A. victoria*. The expression signal of this gene was adapted to lactococci that could then be detected by microscopy with a GFP filter. This allows the detection of lactococci in the DT better than other fluorescent dyes. Lastly, only intact cells are detected, since GFP diffuses rapidly in the DT content after cell lysis. A similar strategy was described previously in order to count viable but not cultivable *Salmonella typhi* in the environment (5). Since we were not able to detect a significant difference between the numbers of viable and intact cells, we deduced that the viable bacteria were rapidly lysed in the duodenum. Moreover, when a large population of *L. lactis* is mixed with the diet, massive lysis occurs, allowing the release of cell content in the duodenum.

Perspectives. This work shows that *L. lactis* should be genetically optimized according to the segment in the DT where the molecule will be targeted. For example, digestive enzymes such as lipase or lactase have to be liberated in the duodenum, and they should be produced intracellularly before the bacteria are ingested, since the amount of protein synthesized in this part of the DT will be not significant. Enzyme-loaded *L. lactis* cells could thus be good vectors to deliver proteins in the duodenum, where the bacteria undergo lysis. By contrast, molecules which have to be targeted to the Peyer patches in the ileum should be produced and secreted in situ, since no significant cell lysis seems to occur in this compartment and since *L. lactis* cells are metabolically active in this compartment.

In this work, we used different luminescent markers to study the fate of a specific cell population. The acidity and activity of trypsin appeared to be very important in the stomach and in the duodenum, respectively. This may guide further work to produce more resistant or sensitive *L. lactis* strains for specific uses. Lastly, this methodology could be adapted not only to other bacteria to target molecules in the DT but also to food strains that are believed to have a beneficial effect on human health.

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