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Survival of *Lactobacillus casei* in the Human Digestive Tract after Consumption of Fermented Milk

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A human trial was carried out to assess the ileal and fecal survival of *Lactobacillus casei* DN-114 001 ingested in fermented milk. Survival rates were up to 51.2% in the ileum and 28.4% in the feces. The probiotic bacterium has the capacity to survive during its transit through the human gut.

The probiotic strain *Lactobacillus casei* DN-114 001 (CNCM number I-1518) reduces the frequency or the duration of episodes of acute diarrhea in young children (20, 21), increases the lactobacillus concentration in the gut microbiota of infants (10), and can modulate ex vivo production of proinflammatory cytokines in Crohn's disease (4). Using mouse models harboring human microbiota, our group recently established that this bacterial strain can initiate new protein synthesis during transit, suggesting that the bacterium is metabolically active (18, 19).

In the present study, a human trial was carried out to assess the survival of *L. casei* DN-114 001 by culture analysis of ileal and fecal samples from healthy subjects consuming fermented milk. The test product (supplied by Danone Vitapole, Palaiseau, France) consisted of yogurt cultures (*Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus*) supplemented with a rifampin-resistant spontaneous variant of *L. casei* DN 114 001, here referred to as strain DN-114 001^{Rif}. This stable variant was isolated according to previously described methods (14) and led to the same growth kinetics and organoleptic properties as the original strain.

Ten volunteers (eight males and two females) with a median age of 25.5 years (range, 22 to 38 years) were included in the study after giving their written consent and were remunerated for their participation in the trial. They had no history of gastrointestinal disorders, no antibiotic treatment during the 2 months preceding the trial, and no laxative treatment for the week prior to the study. During the whole investigation period, the only restriction with regard to diet was the exclusion of fermented dairy products. The study was approved by a local Institutional Ethics Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Necker) and was conducted at Necker Hospital, Paris, France. A blind review of data was performed to identify major protocol deviation and to define intention-to-treat and per-protocol populations. In per-protocol population analysis, all patients who had failed to complete the whole study protocol were excluded. Discontinuous variables were compared using the Wilcoxon rank test. For multiplicity of significance testing in the main analysis, the homogeneity of the two groups was tested using the chi-square test. The confidence interval was calculated as 95% (two tailed). The significance level used was 5%.

The trial consisted of two distinct steps, with an 8-day washout period between steps. The first step corresponded to the ileal-survival study, while the second focused on the analysis of *L. casei* DN-114 001^{Rif} fecal survival.

Design of the ileal-survival study. Seven out of 10 volunteers consented to intestinal intubation, which was performed after a 7-day period during which fermented dairy products were excluded from the diet. Each subject was nasally intubated with a triple-lumen weighed tube (Marquat SA, Boissy-Saint Léger, France), and once the pylorus was passed, a bag placed at the distal end of the first lumen was inflated in order to hasten tube progression. When the bag had reached the cecum, as confirmed fluoroscopically, subjects were asked to stay in a semirecumbent position. The second lumen was used to sample the ileal contents 35 cm above the ileocecal junction, while the third lumen, 25 cm proximal to the aspiration port, was used for infusion of polyethylene glycol (PEG).

On the morning of the following day, an infusion of 10 g PEG 4000 in 154 mmol NaCl · liter^{-1} at 37°C was started at the rate of 2 ml · min^{-1}. After 1 h of equilibration, fasting subjects ingested a standard meal (125 g mashed potatoes, 1 hard boiled egg, 30 g jam, 100 g bread, 30 g butter, 10 g sugar, 250 ml tea or coffee, and a portion of melted cheese) and 3 bottles (3×100 ml in a single dose) of fermented milk containing about 10^8 CFU · ml^{-1} of *L. casei* DN-114 001^{Rif} and 10^6 spores per ml of thermoresistant *Bacillus stearothermophilus* (Merck, Darmstadt, Germany) as a transit marker (15). Since the tube failed to go below the jejunum level in three volunteers, only four subjects were subsequently analyzed (the ileal per-protocol population). The ileal contents were continuously collected on ice by manual aspiration, with the aim of collecting as much

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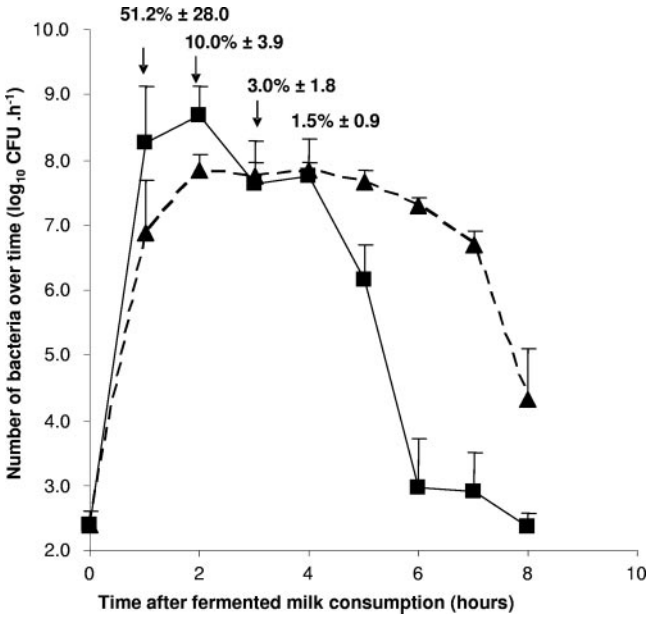

FIG. 1. Transit of *L. casei* (solid line) and spores (dashed line) in the ileal lumens of four volunteers after ingestion of a meal with 300 ml of fermented milk containing *L. casei* (10.9 ± 0.5 $\log_{10}$ CFU per meal) and spores (8.4 ± 0.5 $\log_{10}$ CFU per meal). The results are presented as the number of bacteria transiting over time (mean plus standard error of the mean; $n = 4$). The percentages indicated at the different collection times correspond to *L. casei* apparent survival based on the ratio between the *L. casei* organisms and the spores (Table 2 shows the calculation method). When the percentage is not indicated, it is below 0.1%.

fluid as possible over the 8 h following ingestion of the meal. The aspirated samples were pooled into 1-h aliquots, which were processed immediately for bacterial analysis. The remaining fluids were stored at −20°C for subsequent measurement of the PEG concentrations by means of turbidimetry (11), making it possible to calculate the population of bacteria transiting over time (22). For bacterial enumeration, 10-fold serial dilutions of homogenized ileal fluids were prepared, and each dilution was plated on Man-Rogosa-Sharpe agar medium containing 100 $\mu g \cdot ml^{-1}$ rifampin. The plates were incubated anaerobically at 37°C for 2 days before *L. casei* DN-114 001^{Rif} CFU were counted. *B. stearothermophilus* spores were germinated and enumerated as previously reported (6).

Design of the fecal-survival study. After a 7-day period during which fermented dairy products were excluded from the diet, the 10 initial volunteers were enrolled in the second step of the trial (the fecal intention-to-treat population). No *L. casei* DN-114 001^{Rif} bacteria could be recovered from stools at the end of this washout period.

Each volunteer was asked to consume three bottles (3 × 100 ml) of the test product daily (1 bottle at each meal) for an 8-day period. As for the first step of the trial, 10^8 spores of *B. stearothermophilus* were added to each dose of the test product for the last 3 days of consumption. Stools were collected twice during the ingestion period (the fourth and eighth days) and twice after completion of intake of the test product (the third and seventh days after consumption ceased). The stool samples were processed within 30 min for bacterial enumeration according to the method described for ileal samples. Survival was calculated for samples obtained at the end of the consumption period, at which time full data were available for only six volunteers (four volunteers had missing or invalid data).

***L. casei* recovery from ileal fluid.** A peak in the population of *L. casei* DN-114 001^{Rif} was observed in the ileal fluid during the 3 hours after volunteers consumed the test meal and the single dose of fermented milk (Fig. 1). In contrast, the transit marker persisted longer, reaching a plateau from 2 to 6 h after ingestion. The highest concentration of *L. casei* DN-114 001^{Rif} detected in this compartment represented 10 to 100 times more bacteria than the resident microbiota (3), and it largely exceeded the concentration of probiotics that was previously suggested by some authors for the recovery of a clinical effect (at least 6 $\log_{10}$ CFU $\cdot$ ml^{-1} in the small bowel, according to Marteau et al. (15). Based on PEG measurements (Table 1), the total recovery of *L. casei* DN-114 001^{Rif} in the ileum over the entire 8-hour period was estimated at 9.2 ± 0.5 $\log_{10}$ CFU, corresponding to around 3.6% of the total ingested quantity.

The apparent survival of *L. casei* DN-114 001^{Rif} was greater during the first 2 h of collection (51.2% to 10.0%) than what had been previously reported for other lactobacilli in the ileum (e.g., 0.5% for *Lactobacillus fermentum* KLD [24]; 1.5% for a commercial strain of *Lactobacillus acidophilus* [16]) or in the small intestine (e.g., 7% and 11.8% for *Lactobacillus plantarum* NCIMB 8826 [24] and for *Lactobacillus salivarius* UCC118 [5], respectively).

***L. casei* survival in stools.** In the stool-survival step of the trial, fermented-milk intake was continued long enough for *L. casei* to reach a steady state. Indeed, a plateau of about 7.6 $\log_{10}$ CFU $\cdot$ g^{-1} of stool was observed from 4 to 7 days following the fermented-milk consumption, while this level decreased rapidly after discontinuation (data not shown). The fecal concentrations of *L. casei* DN-114 001^{Rif} reported here are substantially higher than the concentration of *L. casei* strain Shirota, estimated at 7 $\log_{10}$ CFU $\cdot$ g^{-1} of feces with a similar inoculum (25), suggesting lower survival abilities of the latter strain. The fecal population of *L. casei* DN-114 001^{Rif}, corresponding to about 0.1% of the autochthonous microbiota, may be a key factor in its probiotic activities (3, 15), since it has

TABLE 1. Bacterial recovery from ileal fluid collected during 8 h following ingestion of a standard meal and 300 ml of the test product[a]

Inoculum	Total ingested ($\log_{10}$ CFU)	Total recovered ($\log_{10}$ CFU)	% Recovery based on PEG measurements[b]
Spores	8.8 ± 0.1	8.5 ± 0.2	61.7 ± 22.5
L. casei organisms	10.9 ± 0.2	9.2 ± 0.5	3.6 ± 1.8

[a] The study involved seven human volunteers (the ileal intention-to-treat population). Only four volunteers with the triple-lumen tube placed in the correct position were analyzed (the ileal per-protocol population). The results are presented as the mean ± standard error of the mean ($n = 4$).

[b] The ileal flow rate (IFR) was first calculated from PEG dilution values according to previous studies (16, 24, 22) as follows: IFR ($ml \cdot h^{-1}$) = (PEG perfusion flow rate) × (PEG concentration in the perfusion fluid)/(PEG concentration in the ileal fluid). Total counts of bacteria (or spores) transiting at the ileum from 1 to 8 h after ingestion of the meal were then estimated by summing up the counts for each 1-hour period calculated as follows: IFR × (*L. casei* DN-114 001^{Rif} or spore concentration in a 1-hour sample). The percentages recovered were finally obtained by dividing the total number of bacteria (or spores) recovered from the ileal content by the total number of ingested bacteria (or spores).

TABLE 2. Bacterial recovery from fecal samples collected on day 7 of the experiment[a]

Inoculum	Total ingested[b] (log_{10} CFU of fermented milk meal)	Total recovered (log_{10} CFU per 100 g of daily stools)	*L. casei* % apparent survival based on ratio between *L. casei* and spores[c]
Spores	8.8 ± 0.1	8.3 ± 0.2	
L. casei organisms	10.7 ± 0.1	9.7 ± 0.2	28.4 ± 7.0

[a] At day 7, *L. casei* organisms and spores had been consumed for 7 and 3 days, respectively. The study involved 10 human volunteers (the fecal intention-to-treat population), but analyses were made for only 6 volunteers (the fecal per-protocol population; 4 volunteers had missing or invalid data). The results are presented as the mean ± standard error of the mean ($n = 6$).

[b] Volunteers consumed 300 ml of fermented milk.

[c] Calculated as (*L. casei* DN-114 001^{Rif} concentration in a sample/spore concentration in the same sample)/(*L. casei* DN-114 001^{Rif} concentration in the ingested product/spore concentration in the ingested product), as previously described (7).

been demonstrated that probiotic transit can be associated with transient variations of the total population of lactobacilli, enterococci, or bifidobacteria in the microbiota (5, 13, 23), as well as with fluctuations such as beta glucuronidase activity or short-chain fatty acid concentrations (9, 13, 23).

The apparent *L. casei* DN-114 001^{Rif} survival was approximately 28.4% in the feces (Table 2). The difference between apparent survival rates observed in the ileum and in the feces might be related to the ileal sampling procedure. Indeed, we suspect that secretions, such as bile or defensins (8), could have affected the survival of *L. casei* DN-114 001^{Rif} during the 1-h period separating the beginning of sampling and sample quantification. The apparently higher fecal survival could also be explained by *Lactobacillus* multiplication in the colon, as previously suggested (1, 12). Since the consumption protocols were different for ileal (300 ml once) and fecal (100 ml three times a day for 8 days) studies, a cumulative effect could be considered in the fecal experimentation. According to several studies, some *Lactobacillus* strains may attach transiently to the ileal mucosa (2, 17). Thus, a longer stay by lactobacilli consumed during the previous days could account for the apparently higher survival rate measured in the feces.

Conclusions. The present trial led us to conclude that *L. casei* DN-114 001^{Rif} survives well during gastrointestinal transit. In the ileum, the high population level of *L. casei* DN-114 001^{Rif} might be compatible with a probiotic effect. In the feces, it corresponded to the subdominant population (3).

The questions as to whether *L. casei* DN-114 001 is metabolically active and which functions are specifically activated in the human gut still remain to be answered in order to understand the digestive tract adaptation and mechanisms of action of this probiotic.

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