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Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* survive gastrointestinal transit of healthy volunteers consuming yogurt

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Abstract

To date, there is significant controversy as to the survival of yogurt bacteria (namely, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) after passage through the human gastrointestinal tract. Survival of both bacterial species in human feces was investigated by culture on selective media. Out of 39 samples recovered from 13 healthy subjects over a 12-day period of fresh yogurt intake, 32 and 37 samples contained viable *S. thermophilus* (median value of 6.3×10^4 CFU g^{-1} of feces) and *L. delbrueckii* (median value of 7.2×10^4 CFU g^{-1} of feces), respectively. The results of the present study indicate that substantial numbers of yogurt bacteria can survive human gastrointestinal transit.

Keywords: Human trial; *Lactobacillus delbrueckii*; *Streptococcus thermophilus*; Survival; Yogurt

1. Introduction

Yogurt symbiosis is believed to exert beneficial effects on human health. For instance, the role of yogurt bacteria in the reduction of lactose intolerance has been thoroughly studied in humans and is now well established [1–6]. A recent review proposed that fresh yogurt preparations containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* could now be considered probiotic products because they confer health benefits to the host [7]. Otherwise, it is generally demanded that beneficial bacteria be viable in the digestive tract for being recognized as probiotics. Few studies have investigated survival of yogurt bacteria during gastrointestinal transit of human and, to date, results remain rather conflicting. In a recent work, del Campo et al. [8] failed to detect the presence of *S. thermophilus* or *L. delbrueckii* in the feces of 96 volunteers following a 2-week period of yogurt intake (using either culture or specific PCR). In contrast, other authors have succeeded in detecting viable *L. delbrueckii* (but not *S. thermophilus*) in the feces of 6 out of 10 subjects ingesting yogurt [9].

The goal of the present study was to assess the survival of *S. thermophilus* and *L. delbrueckii* by culture analysis of feces from healthy subjects over a 12-day period of yogurt intake. Thirteen 25- to 45-year-old

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volunteers (7 males and 6 females) were included in the study after giving their written informed consent. They had no previous history of digestive surgery and were not subject to chronic diarrhoea or constipation, colonic inflammatory syndrome, allergy or intolerance to dairy products. Also, they had not received any antibiotic treatment during the 3 months preceding the study.

2. Materials and methods

The trial consisted of two consecutive periods. For an initial 15-day period, fermented milk and cheese products, including fresh yogurt, were excluded from the normal diet of the volunteers. One stool sample was obtained from each individual in the last 2 days of this period. During the following 12-day period, volunteers observed the same diet restrictions but were asked to eat 3×125 mL of fresh yogurt daily. Three stool samples were recovered from each subject in the last 5 days of this second period. Bacterial starters used for yogurt preparation consisted in two spontaneous rifampicin and streptomycin resistant variants of *S. thermophilus* S85 and *L. delbrueckii* subsp. *bulgaricus* S85. These variants lead to the same microbiological, technological and organoleptic properties of yogurt as the original strains. Fresh yogurt manufactured with these strains contained 7.8×10^8 CFU mL^{-1} viable *S. thermophilus* and 7.5×10^8 CFU mL^{-1} viable *L. delbrueckii*. Thermoresistant *Bacillus stearothermophilus* spores (Merck, Darmstadt, Germany) were also added to yogurt at 2.3×10^5 CFU mL^{-1} during the fabrication process. They were used as an inert transit marker as previously reported [10,11] and to check product consumption by the volunteers. After yogurt production, bacterial counts did not vary substantially for at least 1 month, ensuring intake of viable microorganisms at constant concentrations throughout the study.

Fecal samples obtained from each volunteer were analyzed within 2 h of stool emission. For each sample, an aliquot of 1 g was homogenized in 10 mL casein-yeast extract medium [12] and 10-fold serial dilutions were prepared. Dilutions up to 10^{-8} of the initial fecal suspension were plated on M17-agar plates containing 1.000 μg mL^{-1} streptomycin and 100 μg mL^{-1} rifampicin for selective recovery of *S. thermophilus*. MRS-agar plates containing the same antibiotic concentrations were used for selective recovery of *L. delbrueckii*. Plates were incubated anaerobically at 42 °C for at least 48 h before enumeration of colonies. Using this protocol, the detection limit for *S. thermophilus* or *L. delbrueckii* in fecal samples was 10^2 CFU g^{-1} of feces. *B. stearothermophilus* spores were enumerated from fecal dilutions according to the previously described procedure [10]. Preliminary experiments confirmed that these selective media and growth conditions make it possible to unequivocally discriminate between the two bacterial strains of yogurt and completely prevent the growth of any other microbial species from fecal microbiota (data not shown).

3. Results and discussion

All the fecal samples obtained during the first period of the trial were analyzed first. No colony was recovered from selective plates, indicating the absence of antibiotic resistant bacteria in the fecal microbiota of all subjects before yogurt intake. The 13 volunteers then proceeded into the second period of the trial. As a result of yogurt consumption, *B. stearothermophilus* spores were present in the three samples obtained from each of the 13 volunteers (Table 1). Spore levels varied within a narrow range and were recovered at a mean value of 6.1 $[\pm 2.4] \times 10^6$ CFU g^{-1} of feces ($n = 39$). These levels were remarkably stable within individuals and very similar between subjects.

During the period of intake, viable *S. thermophilus* cells were recovered from 32 out of 39 fecal samples (82%) at levels between 4.0×10^2 and 3.5×10^6 CFU g^{-1} of feces, with a median value of 6.3×10^4 CFU g^{-1} of feces. Similarly, 37 out of 39 fecal samples (95%) were positive for the detection of viable *L. delbrueckii*. Bacterial levels varied between 5.0×10^2 and 2.1×10^7 CFU g^{-1} of feces with a median value of 7.2×10^4 CFU g^{-1} of feces. In subject 07, the first sample contained none of the two yogurt bacteria in a viable state but each of the other two samples contained either viable *S. thermophilus* or viable *L. delbrueckii*. None of the three samples of subject 09 contained viable *S. thermophilus*, but viable *L. delbrueckii* was present in all of them. Finally, only a single sample out of 39 (3%) was deficient in both viable *S. thermophilus* and *L. delbrueckii*.

Although molecular techniques are highly sensitive and specific for detection of bacterial DNA, classical

Table 1
Recovery of *S. thermophilus*, *L. delbrueckii* and spore marker from feces of yogurt consumers

	Number of volunteers whose feces contained viable microorganisms from yogurt		
	S. thermophilus	*L. delbrueckii*	*B. stearothermophilus* spores
Number of detections per individual			
Three times	9	12	13
Twice	2	0	0
Once	1	1	0
Never	1	0	0
Total of volunteers	13	13	13

culture techniques remain essential for the assessment of cell viability. Nevertheless, the use of non-selective culture methods for detection of viable *S. thermophilus* and *L. delbrueckii* from yogurt in human feces is inadequate since bacteria from the resident microbiota may also grow on plates. Callegari et al. [9] reported that they were unable to recover *S. thermophilus* colonies because other cocci from the human microbiota were too numerous on readable plates. Recently, del Campo et al. [8] attempted to use specific-PCR to identify *S. thermophilus* or *L. delbrueckii* colonies grown on MRS or M17 plates previously spread with fecal samples. However, the accuracy of the method used by these authors is likely to be poor, since a limited number of colonies were PCR tested and the criterion for PCR testing was morphological resemblance to usual *S. thermophilus* or *L. delbrueckii* colony morphology. In the present study we took advantage of spontaneous antibiotic resistant variants for the specific detection of viable *S. thermophilus* and *L. delbrueckii* in the feces of human volunteers consuming yogurt. By preventing growth of any other microorganism on plates, this method allowed us to exclusively enumerate yogurt bacteria. This strategy may account, at least in part, for the significantly higher amounts of viable yogurt bacteria we detected in most volunteers compared to the previous findings of other authors who enrolled numerous subjects [8]. The discrepancies between our results and those of del Campo et al. [8] may also result from differences in the trial protocol. Our analysis was performed on fresh fecal samples collected during the period of yogurt intake, while they analyzed samples taken after the end of the consumption period. These authors did not otherwise indicate how long after the end of yogurt ingestion stool samples were obtained. With respect to potential probiotic effects on subjects consuming yogurt, data on bacterial survival after the end of the consumption period sound meaningless.

The significant recovery of viable *S. thermophilus* and *L. delbrueckii* in human feces is consistent with previous data on the survival of yogurt bacteria in the upper compartments of human digestive tract [4,13,14]. Together with studies reporting metabolic activity of yogurt bacteria in the digestive tract of animal models and humans [14,15], these data on the viability of *S. thermophilus* and *L. delbrueckii* during gastrointestinal transit in humans provides additional evidence of the ability of yogurt symbiosis to exert probiotic effects.

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