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Streptococcus thermophilus Is Able To Produce a β-Galactosidase Active during Its Transit in the Digestive Tract of Germ-Free Mice

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This work presents data on the application of a bacterial luciferase used to monitor gene expression of *Streptococcus thermophilus* in the digestive tract. The main result is that the bacterium was able to produce an active β-galactosidase in the digestive tract, although it did not multiply during its transit. This production was enhanced when lactose (the inducer) was added to the diet.

Lactase deficiency is a widespread problem occurring in approximately 70% of the world's population (17). Consumption of milk by lactase (β-galactosidase)-deficient individuals results in the underabsorption of lactose. This in turn often results in abdominal pain, diarrhea, and flatulence (1). Studies have shown that lactose digestion improves with the ingestion of yogurt compared to the ingestion of heated yogurt or milk (9, 10, 13, 14, 16, 18). These studies suggest that the beneficial effect could have occurred in the digestive tract (DT) after the consumption of the yogurt. The live bacteria present in yogurt may continue to produce β-galactosidase in the intestinal tract.

The aim of the present study was to determine if *Streptococcus thermophilus* is able to produce an active β-galactosidase during its transit in the DT. The *S. thermophilus* lactose (*lac*) operon contains the genes that encode a lactose permease (*lacS*) (12) and a β-galactosidase (*lacZ*) (15) for the transport and the hydrolysis of lactose, respectively. To study the expression of the bacterial β-galactosidase during the transit of *S. thermophilus* in the DT, the luciferase genes of *Xenorhabdus luminescens* (5, 8) were fused to the *lac* promoter. Luciferase is a well-established reporter gene system which has already been used to investigate *Lactococcus lactis* gene expression in the DTs of mice and rats (2, 3).

S. thermophilus strains were grown at 42°C in M17 with 0.5% lactose or with 0.5% glucose supplemented with erythromycin (5 μg/ml). Thermoresistant *Bacillus subtilis* spores were used as a transit marker and germinated at 60°C in G-spore medium (4). Three PCR fragments belonging to the *S. thermophilus* S85 *lac* operon were cloned into the integrative plasmid pil4267 (J. Anba, unpublished data) in front of the luciferase genes to give pFBI3 (carrying the 0.8-kb *lac* promoter region), pFBI4 (carrying a 0.74-kb fragment internal to the *lacZ* gene), and pFBI6 (carrying a 0.8-kb fragment internal to the *lacS* gene). These plasmids were integrated by homologous recombination into the chromosome of the *S. thermophilus* S85 strain grown at 42°C to produce FBI3, FBI4, and FBI6, respectively (Fig. 1). In FBI3, the integration of the plasmid duplicates the *lac* promoter so that the strain had a wild-type phenotype (same β-galactosidase activity and same growth rate in various media). In this strain, the luciferase genes became a functional part of the *lac* operon and were similarly regulated. The luciferase activity of FBI3 (estimated as described by Corthier et al. [2]) was enhanced nine times in M17-lactose compared to M17-glucose (Fig. 2). Moreover, in M17-lactose, the FBI3 strain produced light at the end of the exponential phase. These results are consistent with those described by Poolman et al. (11) and Gunnewijk and Poolman (6, 7). In FBI4 and FBI6, the integration of the pFBI4 and pFBI6 plasmids by simple crossing over interrupted the *lacZ* and *lacS* genes, respectively, leading to erythromycin-resistant recombinants devoid of β-galactosidase activity. Neither the FBI4 strain (devoid of functional LacZ) nor the FBI6 strain (devoid of functional LacS) was able to grow in milk and metabolize lactose, as expected for such mutants.

Six male adult germfree C3H/He mice, reared in sterile Trexler plastic isolators (La Calhène, Vélizy, France), received lactose (4.5% [wt/vol]; this concentration is found in milk and some yogurts) as a drinking solution or water as a control. They then received 0.5 ml of an *S. thermophilus* FBI3 culture (inoculum of 10^8 CFU/ml) mixed with *B. subtilis* spores (inoculum of approximately 10^8 CFU/ml) by oral gavage. Mice feces were collected at various times after inoculation, weighed, and diluted (1/10) in sterile water. Luciferase activities and *S. thermophilus* and *B. subtilis* counts were estimated from these dilutions. The luciferase activities per *S. thermophilus* organism, calculated as previously described (2), were enhanced 10 times when mice received lactose (4.5%) as a drinking solution compared to water (Fig. 3). As observed in vitro (6, 11), lactose

TABLE 1. Determination of the quantity of lactose ingested by germfree mice and excreted in the feces in 24 h

Expt[a]	Lactose (g)	
	Ingested	Excreted in feces
I	0.00 ± 0.00	0.01 ± 0.00
II	0.32 ± 0.01	0.31 ± 0.01
III	0.32 ± 0.01	0.14 ± 0.01

[a] Mice received either water (experiment I) or lactose (4.5%) (experiments II and III) as a drinking solution and were force fed with an *S. thermophilus* FBI3 culture (experiment III).

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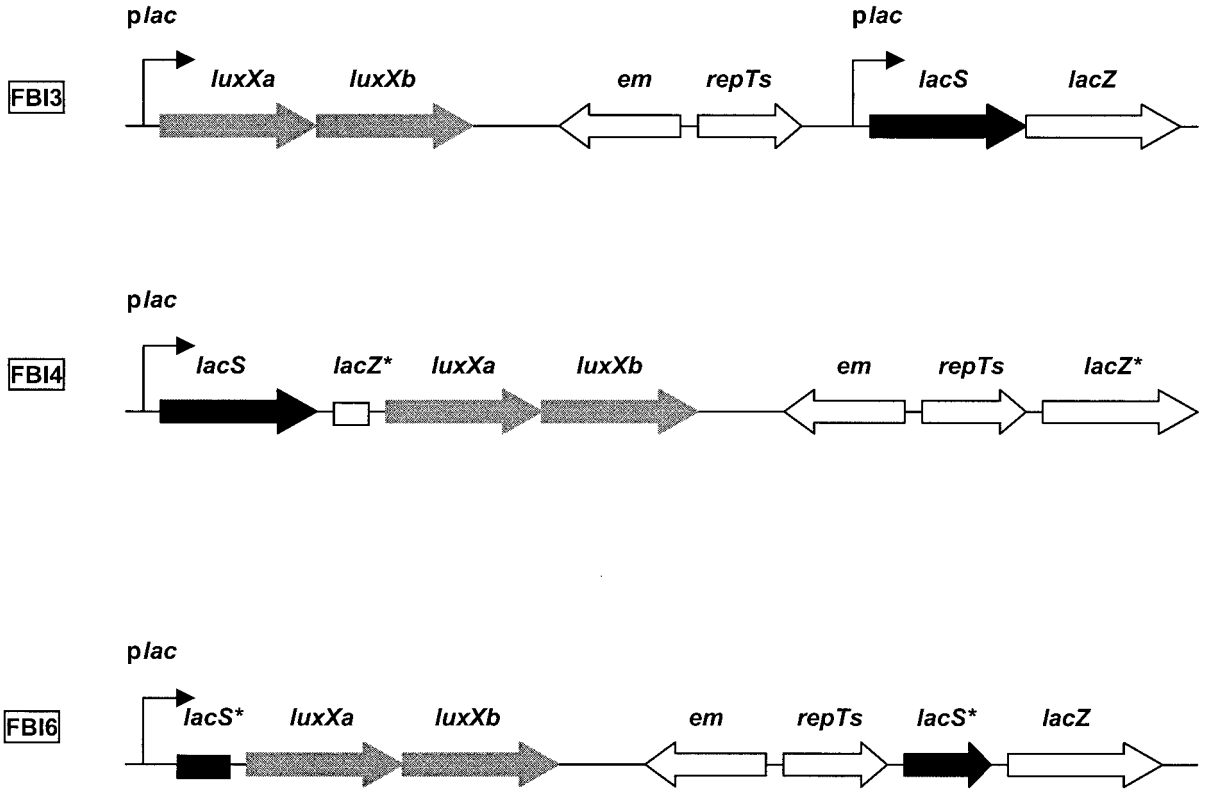

FIG. 1. Chromosomal constructions obtained. FBI3, *lac* operon intact; FBI4, inactivation of *lacZ*; FBI6, inactivation of *lacS.* P*lac*, promoter of the *lac* operon; *lacS*, gene encoding lactose permease; *lacZ*, gene encoding β-galactosidase; *em*, gene encoding resistance to erythromycin; *luxXa* and *luxXb*, genes encoding the *X. luminescens* luciferase; *repTs*, thermosensitive replicon.

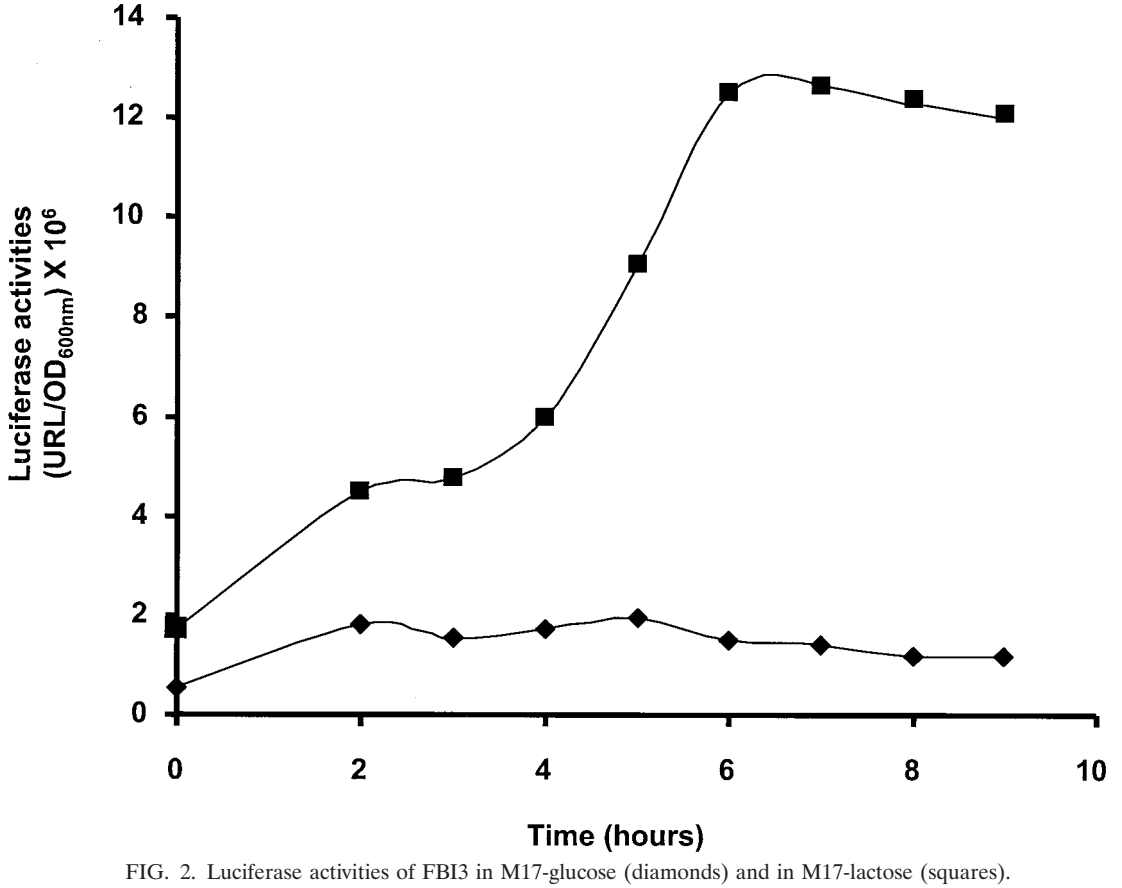

FIG. 2. Luciferase activities of FBI3 in M17-glucose (diamonds) and in M17-lactose (squares).

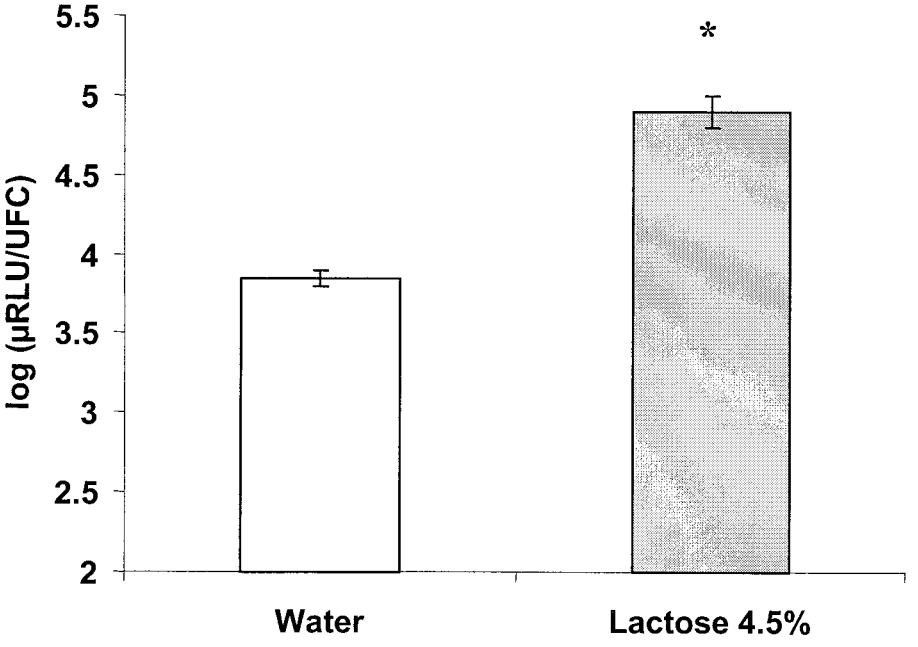

FIG. 3. Luciferase activities in the feces of germfree mice inoculated with FBI3 and receiving either water or lactose (4.5%) as a drinking solution. The luciferase activity of the inoculum was 3.9 log (μ relative light units/unit-forming colony). *, standard deviation < 0.01. Error bars indicate standard deviations.

was an activator of *lac* operon transcription in the DTs of germfree mice. In this experiment *S. thermophilus* did not multiply, did not settle in the DTs of germfree mice, and transited with the diet (Fig. 4). It was eliminated more rapidly than the spores, since 80 h after the inoculation, spores were still detectable in the feces, while the streptococci were under the threshold of detection (data not shown).

The second experiment (with six germfree mice) was designed to determine if *S. thermophilus* was able to digest lactose during its transit in the DTs of mice. Metabolic cages (Tecniplast, Buguggiate, Italy) were used to measure water consumption and collect feces over a 24-h period without urine contamination. No residual lactose was detected in the feces of mice that received water as a drinking solution (Table 1, ex-

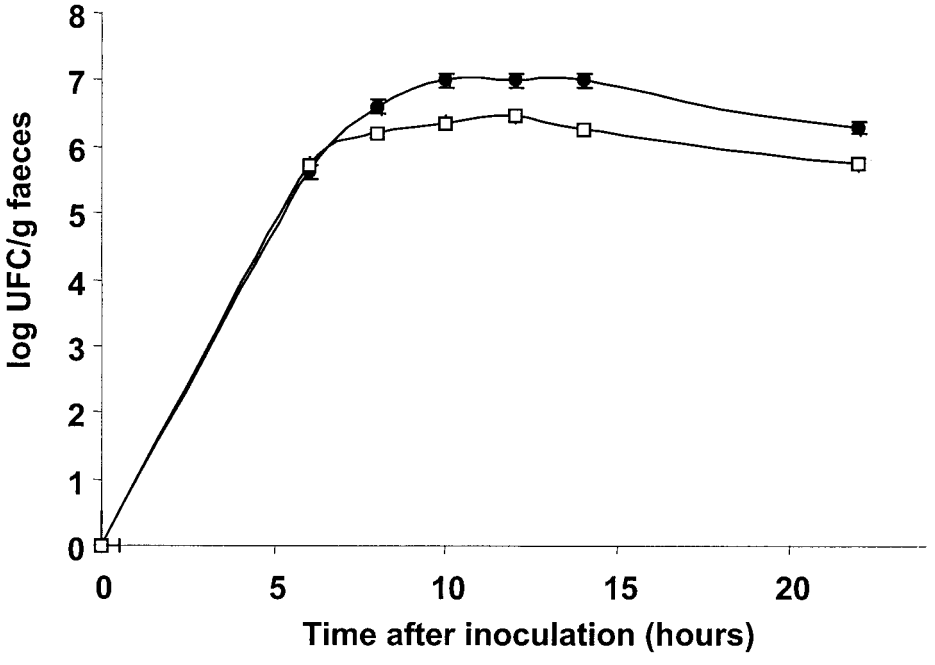

FIG. 4. Enumeration of spores (circles) and *S. thermophilus* FBI3 (squares) in the feces of germfree mice.

periment I). When mice received lactose (4.5%, wt/vol) as a drinking solution, about 0.31 ± 0.01 g of lactose was recovered from feces in 24 h (Table 1, experiment II), suggesting that the mouse lactase was insufficient to digest the lactose absorbed. Oral administration of *S. thermophilus* (inoculum of 10^8 CFU/ml) to mice receiving lactose (4.5% as a drinking solution) resulted in a significant decrease (by 50%) compared to the previous group (Table 1, experiment III). No decrease was observed in mice inoculated with the FBI4 or FBI6 strain (data not shown). This experiment showed that the β-galactosidase produced by *S. thermophilus* is active and is able to hydrolyze lactose in vivo, resulting in an overall reduction of the lactose contents in feces.

Our studies establish, with a genetic approach, that *S. thermophilus* produces a β-galactosidase during its transit and that this enzyme reduces the lactose content in the DT. It reinforces the observation that the bacteria in yogurt must be alive in order to help lactose digestion (10, 13, 14). Our data suggest that yogurt lactic acid bacteria are not "almost dead" in the DT but are still able to respond to diet stimuli and sustain new protein synthesis in order to adapt to environmental conditions. This approach provides new insight into host-microbe interactions and their impact on gastrointestinal health and disease.

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