Carbohydrate Metabolism Is Essential for the Colonization of Streptococcus thermophilus in the Digestive Tract of Gnotobiotic Rats

Muriel Thomas1*, Laura Wrzosek1, Leila Ben-Yahia1, Marie-Louise Noordine1, Christophe Gitton2, Didier Chevret3, Philippe Langella1, Camille Mayeur1, Claire Cherbuy1, Françoise Rul2**

1 Commensal and Probiotics-Host Interactions Laboratory, INRA, UMR1319 Micalis, Jouy-en-Josas, France, 2Peptides and Bacterial Communication Laboratory, INRA, UMR1319 Micalis, Jouy-en-Josas, France, 3 PAPPSO (Plateforme d’Analyse Protéomique de Paris Sud-Ouest) proteomic platform, INRA, UMR1319 Micalis, Jouy-en-Josas, France

Abstract

Streptococcus thermophilus is the archetype of lactose-adapted bacterium and so far, its sugar metabolism has been mainly investigated in vitro. The objective of this work was to study the impact of lactose and lactose permease on S. thermophilus physiology in the gastrointestinal tract (GIT) of gnotobiotic rats. We used rats mono-associated with LMD-9 strain and receiving 4.5% lactose. This model allowed the analysis of colonization curves of LMD-9, its metabolic profile, its production of lactate and its interaction with the colon epithelium. Lactose induced a rapid and high level of S. thermophilus in the GIT, where its activity led to 49 mM of intra-luminal L-lactate that was related to the induction of mono-carboxylic transporter mRNAs (SLC16A1 and SLC5A8) and p27Kip1 cell cycle arrest protein in epithelial cells. In the presence of a continuous lactose supply, S. thermophilus recruited proteins involved in glycolysis and induced the metabolism of alternative sugars as sucrose, galactose, and glycogen. Moreover, inactivation of the lactose transporter, LacS, delayed S. thermophilus colonization. Our results show i) that lactose constitutes a limiting factor for colonization of S. thermophilus, ii) that activation of enzymes involved in carbohydrate metabolism constitutes the metabolic signature of S. thermophilus in the GIT, iii) that the production of lactate settles the dialogue with colon epithelium. We propose a metabolic model of management of carbohydrate resources by S. thermophilus in the GIT. Our results are in accord with the rationale that nutritional allocation via consumption of yogurt alleviates the symptoms of lactose intolerance.

Introduction

Streptococcus thermophilus (S. thermophilus) is a dairy bacterium consumed by Humans for centuries. S. thermophilus is in the European Qualified Presumption of Safety list of food bacteria and is a generally recognized as a safe species (GRAS status); it has a long documented history of safe use in food and its genome is devoided of potential virulence functional genes [1,2]. S. thermophilus is found in numerous cheeses and is one of the two bacteria in yogurt with its obligate partner Lactobacillus delbrueckii sp. bulgaricus (L. bulgaricus). Its high performance in fermenting milk and its resistance to elevated temperatures are two essential properties that accounted for a large utilization of strains of the species S. thermophilus in dairy industry. In addition, S. thermophilus is also recognized as a probiotic and, therefore, used to promote health [3,4,5,6].

Recently, a general claim on yogurt on aiding lactose digestion has been accepted by the European Food Safety Authority. The latter considers that a cause and effect relationship is established between the consumption of live yogurt culture and improvement of lactose digestion in individuals with lactose mal-digestion (EFSA journal, 2010). In Humans, only a low proportion of population retains the capacity to degrade the lactose into adulthood, because the intestinal enzyme lactase-phlorizin hydrolase (more often called lactase or β-galactosidase) activity commonly starts decreasing after the first months or years of life [7,8]. The benefits of yogurt are mainly linked to the metabolic capacities of L. bulgaricus and S. thermophilus that compensate the deficiency of the intestinal enzymes by their own β-galactosidase [9,10,11]. Our recent work brings concordant data about the preponderant role of metabolic adaptations of S. thermophilus in the gastrointestinal tract (GIT) of gnotobiotic rats. Previously, we have shown that S. thermophilus adapts its physiology to GIT by enhancing proteins devoted to carbohydrates metabolism [12]. It is likely that the overall glycolytic metabolic capacity of S. thermophilus is boosted in the GIT, thus explaining how the consumption of this viable microorganism may help the digestion of carbohydrates [9,12]. In yogurt at the end of fermentation process, there is a significant amount of lactose (4–5%) [8,13]. Thus, it could be hypothesized that residual lactose present in yogurt may be deleterious for lactose intolerant individuals; however, the inverse has been observed. The residual lactose in yogurt can be considered as a
prebiotic for people with lactose mal-digestion [14,15,16]. The main mechanisms involved are linked to the adaptation of microbiota activity/composition in the presence of lactose, as illustrating by an enhanced faecal β-galactosidase activity [10,15]. Concurrently, in gnotobiotic rats, β-galactosidase activity of S. thermophilus is higher in the GIT after lactose supplementation [17]. Thus the lactose-related metabolism of viable bacteria present in a final dairy product, and their transit throughout the GIT, is central to better understanding the beneficial effects of fermented products on health [18].

Although S. thermophilus can metabolize a number of different sugars (sucrose, glucose, fructose), lactose remains its favorite substrate. Lactose transport, metabolism, and regulation have been extensively studied in vitro by using different strains of S. thermophilus [19,20] and in regard to their genomic sequences [21,22]. In S. thermophilus, lactose is transported into the cell via the LacS permease which operates as a lactose/galactose antipor or as a galactoside/H+ symporter [23]. LacS activity leads to the release of galactose into the extracellular medium after β-galactosidase has hydrolysed lactose into glucose and galactose. On the one hand, S. thermophilus is the archetype of lactose-adapted bacterium and on the other hand, we have observed that this specialized bacterium revealed a high adaptability of its carbohydrate metabolic pathways to the gut environment [12]. To this end, the objective of this work was to study the impact of lactose and lactose permease on S. thermophilus physiology in the GIT of gnotobiotic rats. We studied the sequenced strain LMD-9 for its colonization capacities, lactate production, proteomic profile and the related host responsiveness when animals received 4.5% of lactose that corresponded milk (Fig. 3). As soon as Ino-LMD9 + lac digestive tract arrest (p27kip1) in colon [12]. SLC16A1 and SLC5A8 mRNAs were respectively 1.8 (≥0.05) and 1.6 (≥0.3) induced in Ino-LMD9 + lac (n = 5) relative to GF + lac (n = 3), p27kip1 protein was 1.8 (≥0.03) induced in Ino-LMD9 + lac in comparison with GF + lac (Fig. 2B), thus confirming that lactate may serve as a biological signal to communicate with host epithelium.

The sugar utilisation of S. thermophilus is preferentially induced in vivo

We compared the proteomic profiles of S. thermophilus, by bidimensional electrophoresis, after growth in milk (inoculum solution) and after 30 days residence in the GIT (faeces of Ino-LMD9 + lac) (Figure S1). Sixty-three proteins displayed different abundances between the 2 conditions: 25 were up-regulated (induction from 2 to 12 fold) and 38 were down-regulated (repression from 2 to 10 fold) in faeces compared to milk (Table 1).

Down-regulated proteins were mainly involved in transcription, translation, nitrogen metabolism (AA biosynthesis, peptidases), and purine and pyrimidine metabolisms. Thus, the S. thermophilus population was maintained at a high level (109 CFU/g) in the GIT even with a low expression level of these pathways, whereas its growth in milk required a high expression and availability of these enzymes.

Sixteen out of twenty-five proteins up-regulated in Ino-LMD9 + lac, compared to milk alone, belong to the carbohydrate metabolism (Figure S1 and Table 1). Of these 16 proteins, five were glycolytic enzymes (PikA, Fba, GapA1, Eno, Pyk) while the others were “upstream” enzymes, (leading to the formation of compounds feeding the glycolytic pathway) and “downstream” enzymes (dissipating the ultimate product of glycolysis, pyruvate). Interestingly, enzymes potentially involved in metabolism of glycerog (GlkP, MalQ), sucrose (PsK, ScbB), galactose (GalT, GalE), maltose (MalQ) or in polysaccharide synthesis (GalE) were significantly more abundant in the GIT than in milk (Figure S1). In comparison with a culture in milk, S. thermophilus + lac diversified its carbon source utilization by inducing the metabolism of alternative sugars in the GIT.

The presence of lactose strengthens the sugar utilisation in vivo

To study the effect of lactose on metabolic pathway of S. thermophilus, we compared two sets of data: i) Primarily listing the proteins differentially expressed in the Ino-LMD (without lactose) digestive tract versus milk and ii) a second listing of proteins differentially expressed in the Ino-LMD9 + lac digestive tract versus milk (Fig. 3). As soon as S. thermophilus was growing in the digestive tract, a large amount of proteins were down-regulated (38 and 41, in presence or absence of lactose, respectively). Thirty four of these
A) Colonization of *S. thermophilus* in mono-associated rats. One ml of *S. thermophilus* LMD-9 strain (6 x 10^8 CFU) was inoculated in germ free rats thus leading to Ino-LMD9 +lac rats, which received water with lactose (4.5% wt/vol). Counts of *S. thermophilus* were obtained by plating on M17 agar lactose the luminal content from ileum, caecum, colon, faeces of Ino-LMD9 +lac (n = 11) rats 30 days after the inoculation.

| Location | CFU/g       |
|----------|-------------|
| ileum    | 1.2 ± 1 x 10^5 |
| cecum    | 9 ± 4 x 10^7  |
| colon    | 1 ± 0.6 x 10^8 |
| feces    | 7.1 ± 4.3 x 10^9 |

B) Log_{10} (CFU/g) of *S. thermophilus* LMD-9 and LMG18311 counted from faecal samples all along the experiment for each mono-associated rat. The colonization curves obtained with LMG18311 and LMD-9 were not significantly different (p = 0.4).

C) Comparison of colonization profile of LMD-9 when rats received lactose (Ino-LMD9 +lac) or not [Ino-LMD9, data from our previous work [12]]. Each column symbolized one rat and X, □, ○, △ represented means of *S. thermophilus* LMD-9 counts obtained from faecal samples recovered, respectively, during the first, second, third and fourth week.

doi:10.1371/journal.pone.0028789.g001

Figure 1. Colonization of *S. thermophilus* in mono-associated rats. One ml of *S. thermophilus* LMD-9 strain (6 x 10^8 CFU) was inoculated in germ free rats thus leading to Ino-LMD9 +lac rats, which received water with lactose (4.5% wt/vol). Counts of *S. thermophilus* were obtained by plating on M17 agar lactose the luminal content from ileum, caecum, colon, faeces of Ino-LMD9 +lac (n = 11) rats 30 days after the inoculation. B) Log_{10} CFU/g of *S. thermophilus* LMD-9 and LMG18311 counted from faecal samples all along the experiment for each mono-associated rat. The colonization curves obtained with LMG18311 and LMD-9 were not significantly different (p = 0.4). C) Comparison of colonization profile of LMD-9 when rats received lactose (Ino-LMD9 +lac) or not [Ino-LMD9, data from our previous work [12]]. Each column symbolized one rat and X, □, ○, △ represented means of *S. thermophilus* LMD-9 counts obtained from faecal samples recovered, respectively, during the first, second, third and fourth week.

doi:10.1371/journal.pone.0028789.g001
intestine-repressed proteins that were both repressed in presence or absence of lactose (Fig. 3) were involved in staple functions as transcription and translation. These results confirmed that a low level of these proteins was sufficient to allow *S. thermophilus* to grow in the GIT and establish a metabolically active population.

Of the intestine-boosted proteins, 7 were involved in glycolysis and over-expressed either in the presence or in the absence of lactose (Fig. 3). General- (DnaK, GroEL) or oxidative-stress and over-expressed either in the presence or in the absence of lactose (Fig. 3) were involved in staple functions as transcription and translation. These results confirmed that a low level of these proteins was sufficient to allow *S. thermophilus* to grow in the GIT and establish a metabolically active population.

In the presence of lactose, 9 supplementary proteins involved in carbohydrate metabolism were over-expressed in the digestive tract (Fig. 3). The presence of lactose allowed *S. thermophilus* to enhance and diversify the metabolism of diverse carbohydrate sources, potentially explaining how the population of *S. thermophilus* overwhelms 10 fold the implanted population in absence of lactose (Fig. 1C). Based on the colonization curve, the proteomic signature of the resultant lactate end-product, the mobilization of the carbohydrate metabolism is determinant and limiting step for the colonization, the growth and the activity of *S. thermophilus* in *vivo* (Fig. 4).

**Effect of lactose permease LacS inactivation on the *S. thermophilus* metabolism**

In *S. thermophilus*, lactose is transported into the cell via the LacS permease which operates mainly as a lactose/galactose antiporter. Considering the central role of *S. thermophilus* carbohydrates metabolism in *vivo*, a negative mutant for the lacS gene was generated by inserting a kanamycine resistance cassette in the lacS gene. The resulting ΔlacS strain was validated in *vivo*, since it did not grow in the presence of lactose in contrast to the wild-type LMD-9 strain (Fig. 5A). In the presence of glucose, sucrose or fructose, ΔlacS and the wild-type LMD-9 strains displayed similar growth rates. In *vivo*, the inactivation of LacS permease impaired the growth in presence of lactose, while preserving the capacity of utilising other carbon sources.

The ΔlacS strain was then challenged in *vivo* for its capacity to colonize the GIT in germ-free rats receiving 4.5% lactose (Ino-LMD9lacS rats). The final level of colonization was identical with ΔlacS and LMD-9 strains, with 1.5 10^9 CFU/g (Fig. 5B). Concordantly, the final amount of faecal lactate was identical in both lots of rats (30±0.5 mM in Ino-ΔlacS lac versus 34.1±7 mM in Ino-LMD9lacS). However, the kinetic of colonisation was altered with ΔlacS strain in the first days after inoculation as the maximal colonization ΔlacS being delayed in comparison with the wild-type strain. In concordance with this delayed colonisation, the L-lactate was undetectable in the first days after inoculation in Ino-LMD9lacS versus GF-lac rats (2-DE analysis).

**Discussion**

Our study demonstrates that the presence of lactose enhanced the fermentative activity of *S. thermophilus* leading to higher level of luminal lactate (×3.5 caecal lactate in Ino-LMD9lac compared to Ino-LMD9 [12]) which subsequent acts to modulate the host epithelium. Therefore, activation of enzymes involved in carbohydrate metabolism constitutes the metabolic signature of *S. thermophilus* in the GIT and allows the dialogue with colon epithelium. Lactose boosted the *S. thermophilus* carbohydrates metabolism that is already high in the digestive tract. *S. thermophilus*, probably because of its intensive use in dairy industry,

| Functional category (number of proteins) | Range of modulation |
|------------------------------------------|---------------------|
| 25 up-regulated proteins in the digestive tract | -Carbohydrate metabolism: 16 \n-Stress and fitness functions: 4 \n-Nitrogen metabolism: 2 \n-Nucleotide metabolism: 3 | 2.1–10.6 \n2.8–12.6 \n2.8–4.6 \n2.2–4.1 |
| 38 down-regulated proteins in the digestive tract | -Transcription/translation: 17 \n-Diverse: 9 \n-Nitrogen metabolism: 6 \n-Nucleotide metabolism: 5 \n-Carbohydrate metabolism: 1 | 2–10 \n2.2–10 \n2.5–5 \n2.5–10 \n2 |

Table 1. Functional distribution of *S. thermophilus* LMD-9 proteins whose abundance changed between the milk inoculum used for gavage and the faeces of Ino-LMD9lacS rats (2-DE analysis).
has evolved by a specialization to lactose degradation with a genome that is considered as “poor”, having lost many genes. The sugar metabolism appears here to have key functions in adaptation to the GIT environment since life cycle of *S. thermophilus* in the GIT relies on carbon metabolism.

Recently, it has been shown that the efficiency of carbohydrates consumption by bifidobacteria correlated with their ability to protect host against infection [25]. From indigenous gut microbiota to sub-abundant transient species, the carbohydrate metabolism is central for bacterial colonization, activity and subsequent dialogue with host [26,27,28,29,30]. Although *S. thermophilus* is rather specialized in the degradation of simple sugars and belongs to numerically minor group of microbiota [31], its carbohydrate metabolism is also prevalent in regulating its own adaptation in GIT and its dialogue with host.

The present work confirmed our previous data stating that lactate produced in Ino-LMD9 by *S. thermophilus* induced both mRNAs SLC16A1/SLC5A8 monocarboxylic transporters and a cell-cycle arrest protein p27kip1 [12]. The levels of induction between Ino-LMD9lac and GFlac were not statistically different to that obtained between Ino-LMD9 and GF [12], indicating that the 3.7 fold highest content of lactate in Ino-LMD9lac (compared to Ino-LMD9) did not correlate with higher stimulation of epithelial transporters and p27kip1. All these results highlight the homeostatic responsiveness of the epithelium, as we have previously observed after intestinal resection [32]. In mono-associated rats, it is likely that a small portion of lactate was shuttled inside colonic cells, while the remaining luminal lactate was excreted in faeces (49.9±5.7 mM caecum versus 34.1±7 mM in faeces). In such models, the major proportion of lactate was excreted, because of the absence of other bacteria that normally inhabit the gut and metabolize lactate. In healthy human adults, lactate produced by gut microbiota is nearly undetectable in faecal samples since it is absorbed by host and also consumed by lactate-utilizing bacteria to produce mainly butyrate [33,34,33,36]. Therefore, one can suppose that, in a context of a complex microbiota, *S. thermophilus* could contribute to microbial ecosystem by favoring lactate-utilizing enzymes or inhibiting low-pH sensitive bacteria [6].

Our study presents data on *S. thermophilus* physiology and demonstrates the flexibility of this bacterium to adapt to digestive environmental constraints and its capacity to diversify the use of alternative sugars. In absence of lactose, *S. thermophilus* develops a capacity to use alternative sugars *in vivo* and this suggests that similar mechanisms occurred in the absence of a functional LacS permease. The presence of lactose enhanced LMD-9 colonization in the GIT, probably by favoring the use of diverse sugars in the GIT via the induction of metabolisms of galactose, sucrose,

**Figure 3. Comparative proteomic analysis (2-DE) of *S. thermophilus* LMD-9.** Cytoplasmic extracts of *S. thermophilus* LMD-9 after passage through the GIT (ino-LMD9lac rats) were compared with those obtained in milk culture (inoculum for gavage). Data obtained in absence of lactose (ino-LMD9) were previously described [12].

doi:10.1371/journal.pone.0028789.g003
maltose, and glycogen. Each metabolic pathway feeds glycolysis, suggesting that *S. thermophilus* metabolism converges to ATP production in the GIT. This also indicates that most of the sugar routes predicted by genome analysis, and so far not yet studied, are probably functional in *S. thermophilus* LMD-9. ΔlacS strain needed about 15 days to stably and maximally implant and thus possibly to set up a metabolic response by using alternative sugars and/or by favoring the entrance of lactose by other unknown transporters. By studying the physiology of *S. thermophilus* in the digestive tract, we also propose that the protein MalQ may be involved in glycogen synthesis rather than in maltose metabolism. In absence of lactose, MalQ was induced in gut and we hypothesized that *S. thermophilus* stocked glycogen in order to face limiting environmental conditions [37]. In presence of lactose, both MalQ and

**Figure 4. Model of carbohydrate metabolism of *S. thermophilus* in the digestive tract.** Carbohydrate metabolism in *S. thermophilus* LMD-9 after its passage through the GIT, A) in presence (Ino-LMD9<sup>Δlac</sup>) or B) in absence of lactose (Ino-LMD9). Up-regulated enzymes in GIT compared to milk are grey shaded. We constructed this model from the present proteomic data and from predictions of metabolism pathways of LMD-9 strain genome. doi:10.1371/journal.pone.0028789.g004

**Figure 5. *S. thermophilus* ΔlacS characterization.** A) *in vitro*: growth of the wild-type (■) and the ΔlacS (□) strains in M17 in presence of different sugars. B) *in vivo*: enumeration of viable wild-type (▲) and ΔlacS (●) strains isolated from faeces of Ino-LMD9<sup>Δlac</sup> (n = 5) and Ino-ΔlacS<sup>Δlac</sup> (n = 5), respectively. doi:10.1371/journal.pone.0028789.g005
GlgP [responsible for glycogen breakdown in bacteria [38]] were induced, suggesting that equilibrium between anabolism and catabolism occurs in S. thermophilus. A similar simultaneous glycogen synthesis and degradation have been described in Corynebacterium glutamicum where glycogen is constantly recycled [39]. All our observations show that MalQ is not involved in maltose metabolism, in concordant with the fact that no MalE transporter is present in the genome of LMD-9 and that genes malQ and glgP (encoding potentially for a glycogen phosphorylase) are an operon in LMD-9. Glycogen could be of prime importance in the colonization, adaptation and survival of S. thermophilus within the GIT, as previously observed for other microorganisms, when present in starvation conditions [37] or during transition between nutrient-rich- and nutrient-poor environments [40].

Our present work sheds new light on the established association of S. thermophilus and lactose by revealing that lactose enhanced S. thermophilus kinetics, level of colonization and fermentative activity in the GIT. Our work suggests that a food product containing both a live S. thermophilus and lactose would favor the colonization and fermentative activity of S. thermophilus in vivo. In this context, our results follow the rationale of previous clinical observations and the nutritional assertion that the consumption of yogurt (containing lactose) could alleviate the symptoms of lactose intolerance.

Materials and Methods

Bacterial strains, media, and inoculating samples

The strains S. thermophilus LMD-9 (ATCC BAA-491, USA), and LMG18311 (BCCM collection, Belgium) were used. Stock cultures of S. thermophilus LMD-9 and LMG18311 were prepared in reconstituted 10% (wt/vol) Nilac skim milk (NIZO, Ede, the Netherlands) as previously described [41]. S. thermophilus monocultures were obtained by inoculating Nilac milk with 10⁶ CFU/ml of stock cultures and incubated at 42°C until pH 5.4–5.5. One ml of culture was used for rat gavage and the remaining aliquots were frozen in liquid nitrogen and stored at −20°C until protein extraction. The cultures were enumerated a posteriori by plating appropriate dilutions on M17 agar lactose (10 g/L) for S. thermophilus. After 16 h (S. thermophilus) incubation at 42°C under anaerobiosis (Anaerocult A, Merck, Darmstadt, Germany), colonies were counted.

μmax determination

Cultures (n = 3) of S. thermophilus strains were performed in M17 supplemented with 10 g/L lactose, glucose, sucrose or fructose; the apparent growth rate (μmax) was defined as the maximum slope of semi-logarithmic representation of growth curves assessed by O.D.660 nm measurements.

Insertional inactivation of S. thermophilus LMD-9 lacS gene

The kanamycin cassette of the plasmid pKa 2000 was PCR-amplified using the Phusion high fidelity DNA polymerase with Apha3-F (5’CCACCGAACCATTGGA3’) and Apha3-R (5’GGTGGAGATTGACTTCAG3’) primers. The 1489 bp DNA fragments flanking the lacS gene were PCR-amplified using the Phusion DNA polymerase, the LMD-9 DNA as a template, and primers LacS-up (5’TATGGTGCCTGCGATGCA3’)/Kana-up-R (5’AGGGGTCCGAGGCTCGGCAGGATTTGATCATGACGTTTGGATTTTCCAT3’) for the upstream fragment and primers LacS-down (5’AACTGGACGAGCCTTTGAA3’)/Kana-down- (5’CTTACCCTATACCTCAATGGTTTGCGTGGGTTATCGTAAATTTCAAGAAAAA3’) for the downstream fragment. The 3’ end of the upstream generated fragment contained a sequence complementary to the 5’ end of the kana cassette whereas the 5’ end of the downstream generated fragment contained a sequence complementary to the 3’ end of the cassette. This allowed joining of these three fragments subsequent to Taq Phusion PCR using primers LacS-up and LacS-down. After purification with a QIAquick PCR purification kit, 500 ng of the resulting 3.4 kb fragment was further used to transform LMD-9 natural competent cells as described by Gardan et al. [42]. Transformants were selected on M17Glc plates with kanamycin and were then checked by PCR using oligonucleotides LacS-up and KanaR. Finally sequencing of the flanking regions was performed to ensure that no unwanted mutations were introduced.

Animals and experimental design

All procedures were carried out in accordance with European and French guidelines for the care and use of laboratory animals. Permission 78–123 is a permit number dedicated to M. Thomas. MICALIS (Microbiologie de l’Alimentation au Service de la Santé) review board specifically approved this study. At the age of 2 months, germ-free (GF) rats (male, Fisher 344) were inoculated either with S. thermophilus LMD-9 (Ino-LMD9lac+, n = 11) or S. thermophilus LMG18311 (Ino-LMG18311lac+, n = 8). 1 ml of a culture of S. thermophilus in Nilac milk (5×10⁶ CFU/ml) was transferred to GF rats by oral gavage. As a control, GF rats were also inoculated with 1 ml of sterile Nilac milk (without bacteria). Following gavage, rats received water enriched with lactose (4.5%/wt/vol). GF and mono-associated rats were housed in sterile Plexiglas isolators (Ingénia, Vitré-sur-Seine, France). All groups of rats received the same standard diet (UAR), which was sterilized by gamma irradiation. Twice a week S. thermophilus was enumerated by plating serial dilutions of the faeces on M17 lactose agar. All rats were euthanized at three months old, 30 days after gavage.

S. thermophilus ΔlacS strain was inoculated in GF rats to obtain Ino-ΔlacSlac+ rats. The ΔlacS inoculum was grown in M17+glucose and bacteria were enumerated by plating serial dilutions of the faeces on M17 glucose agar supplemented with kanamycine (1 mg/ml). Ino-ΔlacSlac+ (n = 5) rats drank 4.5% lactose-enriched water. The presence of kanamycin cassette disrupting the lacS gene was checked by PCR in faeces of Ino-ΔlacSlac+ rats. Ino-ΔlacSlac+ rats were euthanized 18 days after gavage.

Rats were anesthetized with isoflurane and tissues were recovered. The colon was immediately used, for colonic epithelial cell isolation or for histological procedures as it has been described in Cherbuy et al. [43,44].

Western blot analysis

Colonic proteins from isolated epithelial cells were used for Western blot analysis as previously described [24] by using a denaturing (SDS)-polyacrylamide gel. Proteins were analysed using anti-p27kip1 (Santa Cruz Biotechnology; 1/500). GAPDH was used as loading control. Signals imprinted on autoradiography films were quantified by scanning densitometry of the autoradiograph using Biovision 1000 and logiciel bioID (Vilber Lourmat, France).

Dosage of d- and l-lactates

d- and l-lactates were measured in caecal contents and faeces with the Biosente d/L lactic acid enzymatic kits according to the manufacturer instructions (Biosente, Toulouse, France) as also described in Rul et al. [12].
Histology analysis
Colon samples were cut into 2 cm sections, fixed in 4% paraformaldehyde (4 hours, room temperature), dehydrated and embedded in paraffin according to standard histological protocols. Four micrometer sections were mounted on SuperFrost® Plus slides. Slides were stained with Hematoxylin-Eosin-Safran (HES) for histological analysis. Cryo techniques were determined with NDF.view software (Hamamatsu). Only U shaped longitudinally cut crys with open lumina along the cryo axis were analysed. Results were the mean obtained by analysis at least 20 crypts per rat (Ino-LMD9lac, n = 5; Ino-LMG10311lac, n = 3).

RNA isolation and quantitative RT-PCR analysis
Total RNAs were extracted from colon epithelial cells of GF+ (n = 3), and Ino-LMD9lac (n = 5) by the guanidium thiocyanate method. The sk16a1 and sk5αβ mRNA quantification was performed as we have previously described [12].

Bacterial protein extraction, comparative bi-dimensional (2-DE) protein analysis and image analysis
Cytosplasmic proteins were extracted from bacteria as previously described [12] either from milk cultures (n = 3) or faecal samples (5 g of frozen faeces from 3 Ino-LMD9lac rats). A volume of cytosolic fraction corresponding to 250 μg of proteins was treated as previously described [45]. Bi-dimensional (2-DE) protein analysis and image analysis were performed according to Rul et al. [12]. MS analyses were performed using a Voyager-DE-STR (Applied Biosystems, Framingham, USA) on the PAPPSO proteome platform (http://pappso.inra.fr). The proteins were identified using MS-FIT (http://prospector.ucsf.edu).

Statistical analysis
Results are presented as means ± SE for the number of animals indicated. Comparisons of group data between different batches of rats were performed using one–way analysis of variance (ANOVA) followed by Tukey’s student range test where appropriate. Significance was for P value lower than 0.05. Statistical analysis was performed using the JMP® software (version 7, SAS institute INC).

Supporting Information
Figure S1 Fold changes in protein abundance (2-DE) of S. thermophila: LMD-9 between faeces in presence of lactate and lactose-growth phase in milk.

Acknowledgments
We thank Sylvie Miquel and Neil Dufton for fruitful discussions and critical reading of the manuscript. The authors would like to thank the team of animal facilities (ANAXEM team, Micalis) and Stephan Bouet for technical assistance.

Author Contributions
Conceived and designed the experiments: MT CC FR. Performed the experiments: MT LW MLN CG DC CM. Analyzed the data: MT LW CM FR. Contributed reagents/materials/analysis tools: MT LW LBY MLN CG DC CM. Wrote the paper: MT PL CC FR.

References
1. Bolotin A, Quinquis B, Renaud P, Sorokin A, Ehrlich SD, et al. (2004) Complete sequence and comparative genome analysis of the dairy bacterium Streptococcus thermophilus. Nat Biotechnol 22: 1554–1558.
2. Delorne C (2008) Safety assessment of dairy microorganisms: Streptococcus thermophilus. Int J Food Microbiol 126: 274–277.
3. Gueret F, Perdigon G, Corthier G, Salminen S, Kolotzek B, et al. (2005) Should yoghurt cultures be considered probiotics? Br J Nutr 93: 763–766.
4. Pagani C, Nael R, Banias G, Arsenault KO, Pizarro TT, et al. (2010) Probiotics promote gut health through stimulation of epithelial innate immunity. Proc Natl Acad Sci U S A 107: 454–459.
5. Saavedra JM, Alis-Hanna A, Moore N, Volkens RH (2004) Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. Am J Clin Nutr 79: 261–267.
6. Veiga P, Gallini CA, Beal C, Michaud M, Delaney ML, et al. (2010) Bifidobacterium animals subsp. lactis fermented milk product reduces inflammation by altering a niche for coligenic microbes. Proc Natl Acad Sci U S A 107: 18112–18117.
7. Campbell AK, Waud JP, Matthews SB (2005) The molecular basis of lactose intolerance. Sci Prog 88: 157–202.
8. Lomer MC, Parkes GC, Sanders JD (2008) Review article: lactose intolerance in clinical practice—myths and realities. Aliment Pharmacol Ther 27: 93–103.
9. Drouault S, Anba J, Corthier G (2002) Streptococcus thermophilus is able to degrade lactose and galactose, and methylumbelliferyl–β-galactoside, naphthol–AS–beta glucuronide and naphthol–AS–beta sulphone. FEMS Microbiol Lett 94: 149–153.
10. Makarova K, Slesarev A, Wolf Y, Sorokin A, Miroquin B, et al. (2006) Comparative genomics of the dairy bacterium Streptococcus thermophilus revealed by comparative genomic FEBS Microbiol Rev 29: 453–463.
11. Bolotin A, Quinquis B, Renaud P, Sorokin A, Ehrlich SD, et al. (2004) Complete sequence and comparative genome analysis of the dairy bacterium Streptococcus thermophilus. Nat Biotechnol 22: 1554–1558.
12. Rul F, Ben-Yahia L, Chegdani F, Wrzosek L, Thomas S, et al. (2011) Impact of Lactobacillus rhamnosus GG administration on the kinetic mechanism of transport. J Biol Chem 286: 22087–22094.
13. Pochart P, Dewit O, Desjeux JF, Bourlioux P (1989) Viable starter culture, beta-galactosidase activity, and lactose in duodenum after yogurt ingestion in lactase-intolerant subjects. J Appl Microbiol 104: 595–604.
14. Hertzler SR, Huynh BC, Savaiano DA (1995) Colonization adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance. Am J Clin Nutr 61: 232–236.
15. Hertzler SR, Savaiano DA (1996) Colonics adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance. Am J Clin Nutr 64: 232–236.
16. Szlagyi A (2002) Review article: lactose—a potential prebiotic. Aliment Pharmacol Ther 16: 1391–1602.
17. Mayer DD, Drouault-Holovacs O, Ouseer R, Langella P, Ana J, et al. (2006) Beta-galactosidase production by Streptococcus thermophilus is higher in the small intestine than in the caecum of human-microbiota-associated mice after lactose supplementation. Br J Nutr 96: 177–181.
18. Turpin W, Humblot C, Thomas M, Guyot JP (2010) Lactobacilli as multifaceted probiotics with poorly disclosed molecular mechanisms. Int J Food Microbiol 143: 87–102.
19. Van den Bogaard PTC, Kleerebezem M, Kuipers OP, De Vos WM (2000) Control of lactose transport, beta-galactosidase activity, and glycolysis by CcpA in Streptococcus thermophilus: Evidence for carbon catabolite repression by a non-phosphoenolpyruvate-dependent phosphotransferase system sugar. Journal of Bacteriology 182: 5982–5989.
20. Hori T, Hancock F, Fontaine L, Grossard B, Prozzi D, et al. (2005) New insights in the molecular biology and physiology of Streptococcus thermophila revealed by comparative genomics. FEMS Microbiol Rev 29: 453–463.
21. Bolotin A, Quinquis B, Renaud P, Sorokin A, Ehrlich SD, et al. (2004) Complete sequence and comparative genome analysis of the dairy bacterium Streptococcus thermophilus. Nat Biotechnol 22: 1554–1558.
22. Makarova K, Slesarev A, Wolf Y, Sorokin A, Miroquin B, et al. (2006) Comparative genomics of the lactic acid bacteria. PNAS 103: 15611–15616.
23. Foucaud C, Poolman B (1992) Lactose transport system of Streptococcus lactis. J Bacteriol 174: 6035–6038.
24. Cherbuy C, Honvo-Houeto E, Bruneau A, Bridonneau C, Mayeur C, et al. (2010) Microbiota matures colonic epithelium through a coordinated induction of cell cycle-related proteins in gnotobiotic rat. Am J Physiol Gastrointest Liver Physiol 309: G548–G557.
25. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, et al. (2011) Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 469: 543–547.
26. Chang DE, Smallay DJ, Tucker DL, Leatham MP, Norris WE, et al. (2004) Carbon nutrition of Escherichia coli in the mouse intestine. Proc Natl Acad Sci U S A 101: 7427–7432.
27. Denou E, Pridmore RD, Berger B, Panott JM, Arigoni F, et al. (2008) Identification of genes associated with the long-gut-persistence phenotype of the probiotic Lactobacillus johnsonii strain NCC533 using a combination of genomics and transcriptome analysis. J Bacteriol 190: 3161–3168.
28. Marco ML, de Vries MC, Wels M, Molenaar D, Mangell P, et al. (2010) Convergence in probiotic Lactobacillus gut-adaptive responses in humans and mice. ISME J 4: 1481–1484.

29. Marco ML, Peters TH, Bongers RS, Molenaar D, van Hemert S, et al. (2009) Lifestyle of Lactobacillus plantarum in the mouse caecum. Environ Microbiol 11: 2747–2757.

30. Roy K, Meyrand M, Corthier G, Monnet V, Mistou MY (2008) Proteomic investigation of the adaptation of Lactococcus lactis to the mouse digestive tract. Proteomics 8: 1661–1674.

31. Qin J, Li R, Ras J, Arumugam M, Burgeff KS, et al. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464: 59–65.

32. Joly F, Mayeur C, Messing B, Lavergerne-Slove A, Cazals-Hatem D, et al. (2009) Morphological adaptation with preserved proliferation/transporter content in the colon of patients with short bowel syndrome. Am J Physiol Gastrointest Liver Physiol 297: G116–123.

33. Duncan SH, Belenguer A, Holzapfel G, Johnstone AM, Flint HJ, et al. (2007) Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. Appl Environ Microbiol 73: 1073–1078.

34. Hove H, Mortensen PB (1995) Colonic lactate metabolism and D-lactic acidosis. Dig Dis Sci 40: 320–330.

35. Hove H, Rye Clausen M, Broech Mortensen P (1993) Lactate and pH in faeces from patients with colonic adenomas or cancer. Gut 34: 625–629.

36. Munoz-Tamayo R, Larroche B, Walter E, Dore J, Duncan SH, et al. (2011) Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. FEMS Microbiol Ecol 76: 615–624.

37. Wilson WA, Roach PJ, Montero M, Barjoa-Fernandez E, Munoz FJ, et al. (2010) Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbiol Rev 34: 952–985.

38. Alonso-Casajas N, Duvalle D, Viale AM, Munoz FJ, Barjoa-Fernandez E, et al. (2006) Glycogen phosphorylase, the product of the glgP gene, catalyzes glycogen breakdown by removing glucose units from the nonreducing ends in Escherichia coli. J Bacteriol 188: 5266–5272.

39. Seibold GM, Eikmanns BJ (2007) The glgX gene product of Corynebacterium glutamicum is required for glycogen degradation and for fast adaptation to hyperosmotic stress. Microbiology 153: 2212–2220.

40. Bourassa L, Camilli A (2009) Glycogen contributes to the environmental persistence and transmission of Vibrio cholerae. Mol Microbiol 72: 124–138.

41. Herve-Jimenez L, Guillouard I, Guedon E, Gautier C, Boudelhouar S, et al. (2008) Physiology of Streptococcus thermophilus during the late stage of milk fermentation with special regard to sulfur amino-acid metabolism. Proteomics 8: 4273–4286.

42. Gardan R, Beset C, Guillot A, Gitton C, Monnet V (2009) The oligopeptide transport system is essential for the development of natural competence in Streptococcus thermophilus strain LMD-9. J Bacteriol 191: 4647–4655.

43. Cherbuy C, Andrieux C, Houvov-Houacto E, Thomas M, Ide C, et al. (2004) Expression of mitochondrial HMGC0A synthase and glutaminase in the colonic mucosa is modulated by bacterial species. Eur J Biochem 271: 87–95.

44. Cherbuy C, Darcy-Vrilhon B, Morel MT, Pégourié JP, Duée PH (1995) Effect of germfree state on the capacities of isolated rat colonocytes to metabolize n-butyrate, glucose, and glutamine. Gastroenterology 109: 1890–1899.

45. Derzelle S, Bolotin A, Mistou M, Rul F (2005) Proteome analysis of Streptococcus thermophilus grown in milk reveals pyruvate formate-lyase as the major upregulated protein. Appl Environ Microbiol 71: 8597–8605.