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Research Article

RESEARCH ARTICLE

Sucrose metabolism alters *Lactobacillus plantarum* survival and interactions with the microbiota in the digestive tract

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One sentence summary: Impaired sucrose metabolism results in higher levels of *Lactobacillus plantarum* survival in the murine digestive tract and alters *L. plantarum* effects on the gut microbiome.

Editor: Cindy Nakatsu

ABSTRACT

We investigated whether sucrose metabolism by probiotic *Lactobacillus plantarum* influences the intestinal survival and microbial responses to this organism when administered to mice fed a sucrose-rich, Western diet. A *L. plantarum* mutant unable to metabolize sucrose was constructed by deleting *scrB*, coding for beta-fructofuranosidase, in a rifampicin-resistant strain of *L. plantarum* NCIMB8826. The ScrB deficient mutant survived in 8-fold higher numbers compared to the wild-type strain when measured 24 h after administration on two consecutive days. According to 16S rRNA marker gene sequencing, proportions of *Faecalibacterium* and *Streptococcus* were elevated in mice fed the *L. plantarum* Δ*scrB* mutant. Metagenome predictions also indicated those mice contained a higher abundance of lactate dehydrogenases. This was further supported by a trend in elevated fecal lactate concentrations among mice fed the Δ*scrB* mutant. *L. plantarum* also caused other changes to the fecal metabolomes including higher concentrations of glycerol in mice fed the Δ*scrB* mutant and increased uracil, acetate and propionate levels among mice fed the wild-type strain. Taken together, these results suggest that sucrose metabolism alters the properties of *L. plantarum* in the digestive tract and that probiotics can differentially influence intestinal metabolomes via their carbohydrate consumption capabilities.

Keywords: *Lactobacillus plantarum*; obesogenic diet; carbohydrate metabolism; gut microbiome; fecal metabolome; ecological fitness

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INTRODUCTION

Most established probiotic strains possess the necessary metabolic and stress-adaptive capacities to survive gastrointestinal (GI) tract transit and maintain at least temporary residence in the distal intestine (Lebeer, Vanderleyden and De Keersmaecker 2008). Host factors such as diet, health-status, genetics and resident microbial populations are variables that might affect colonization or residence times and could also be responsible for inter-individual differences in probiotic survival and efficacy (Marco and Tachon 2013). Among those variables, we have found that host diet has significant consequences on probiotics in the GI tract (Marco *et al.* 2009; Tachon, Lee and Marco 2014; Yin *et al.* 2017). This possibility is consistent with the established knowledge that diet has both short- and long-term impacts on the indigenous, intestinal microbiome composition and function (Flint, Duncan and Louis 2017). However, a mechanistic understanding of diet-dependent differences in probiotic activity in the intestine remains to be elucidated. This is especially important for individual food components that are present in high quantities and have known relevance to microbial growth and metabolism.

Previous studies have shown that *Lactobacillus plantarum* WCFS1 (a single colony isolate of *L. plantarum* NCIMB 8826) (Kleerebezem *et al.* 2003) survives in significantly higher numbers in the digestive tracts of mice fed a high-fat, high-sucrose containing diet (HFHSD) compared to a low-fat, plant-polysaccharide rich diet (LFPPD) (Tachon, Lee and Marco 2014). This result was confirmed by a diet-switch study in which intestinal persistence and survival in mice was increased during HFHSD feeding periods and reversed when a LFPPD was consumed (Yin *et al.* 2017).

Mono- and disaccharides are the main energy sources in HFHS diets. Although these carbohydrates are easily digested and absorbed in the small intestine, elevated levels of glucose, fructose and sucrose in the diet were shown to affect host gut microbiota composition in rodent models (Licht *et al.* 2006; Noble *et al.* 2017). *L. plantarum*, like other lactic acid bacteria (LAB), is saccharolytic and its ecological success is regarded to be largely dependent on the capacity for rapid fermentation of mono- and disaccharides to lactic acid. Genome-wide transcriptome profiling studies of *L. plantarum* have indicated that this organism alters energy metabolism to consume dietary carbohydrates during passage through human and murine GI tracts (Marco *et al.* 2009, 2010; Tachon, Lee and Marco 2014). *L. plantarum* gene transcripts for beta-fructofuranosidase (ScrB) were significantly elevated in the intestines of both healthy and colitic mice fed a HFHSD (Tachon, Lee and Marco 2014). *L. plantarum* WCFS1 sucrose transport and metabolism genes were also induced in germ-free mice fed a HFHSD (Marco *et al.* 2009). In both of those studies, sucrose accounted 34% of the weight and 29% of the energy in the HFHSD.

We hypothesized that sucrose metabolism is an important ecological fitness determinant of *L. plantarum* in the digestive tract. *L. plantarum* NCIMB8826 and an isogenic *scrB* deletion mutant were measured for their capacity to survive GI tract transit and alter gut microbiota composition and metabolism.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bacterial strains and plasmids used in the study are listed in Table 1. The isolation of a spontaneous rifampicin-resistant mutant of *L. plantarum* NCIMB8826 (NCIMB8826-R) was described previously (Tachon, Lee and Marco 2014). *L. plantarum* was grown statically at 37°C in de Man Rogosa and Sharpe (MRS) medium (BD, Franklin Lakes, NJ, USA) or modified MRS (Lee *et al.* 2015a) containing 2% (w/v) glucose or sucrose. *Escherichia coli* DH5α was grown under aeration at 200 rpm at 37°C in LB broth (Fisher Scientific, Pittsburgh, PA, USA). When appropriate, antibiotics were added to the media at the following concentrations: erythromycin (Sigma-Aldrich, St. Louis, MO, USA), 5 μg/mL and rifampicin (Thermo Fisher Scientific, Waltham, MA, USA), 50 μg/mL. Optical density at 600 nm (OD_{600}) was measured on a BioTek Synergy 2 multi-mode microplate reader (Fisher Scientific, Pittsburgh, PA, USA).

Mutant construction

A *L. plantarum* Δ*scrB* mutant was generated through double-crossover homologous recombination using a suicide vector pRV300 (Leloup *et al.* 1997). For mutant construction, upstream and downstream flanking regions of the *scrB* gene were amplified using primers A/B and C/D, respectively (Table S1, Supporting Information). Resulting PCR products were combined by splicing-by-overlap extension (SOEing) PCR as previously described (Heckman and Pease 2007). PCR products were digested with SalI and SacII (New England Biolabs, Ipswich, MA, USA), ligated into pRV300, and transformed into *E. coli* DH5α to yield pRVscrB. The plasmid was then introduced to *L. plantarum* NCIMB8826-R by electroporation. Erythromycin-resistant mutants were selected and confirmed for plasmid integration by PCR using primers E/F and G/H (Table S1, Supporting Information). Subsequently, Δ*scrB* mutants were identified by a loss of resistance to erythromycin and PCR amplification using primers I/J (Table S1, Supporting Information). A single *scrB* deletion mutant *L. plantarum* LM0187 was used in subsequent experiments.

Mouse study design

All procedures were performed under the protocol approved by the UC Davis Animal Care and Use Committee (protocol # 17899). A total of 37, 6-week-old, female BALB/c mice (Harlan, Livermore, CA, USA) were singly housed and given free access to food and water on a 12 h light/dark cycle. On day 1 of the study, a high-fat, high-sucrose diet (HFHSD; Research Diet D12079B, Research Diet, New Brunswick, NJ, USA) was introduced and fed for the duration of the study.

Starting from day 6, mice consumed PBS (n = 13), 10^9 cells of *L. plantarum* NCIMB8826-R (n = 12) or LM0187 (n = 12) in 50 μL suspensions from the tip of a ball-tipped gavage bulb. This high number of cells was administered in order to be consistent with and to facilitate comparisons to prior studies on *L. plantarum* transit times (Marco *et al.* 2009; Tachon, Lee and Marco 2014; Yin *et al.* 2017). The cell suspensions were administered daily for 2 days (Fig. 1A) (Tachon, Lee and Marco 2014). To prepare *L. plantarum* inoculum for mouse consumption, *L. plantarum* strains were grown in MRS medium until stationary phase was reached (between 14 and 16 h). Cells were collected by centrifugation at 2,057 g for 10 min, washed twice with phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4), and then suspended in a volume of 20 μL PBS (approximately 10^9 cells) for mice to drink from the tip of a gavage bulb following procedures described in Lee *et al.* (2015b). Freshly expelled stools were examined for rifampicin-resistant bacteria prior to and during *L. plantarum* feeding as previously reported

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Table 1. Bacterial strains and plasmids used in the study.

Strain or plasmid	Relevant characteristics	Reference
L. plantarum NCIMB8826-R	Spontaneous rifampicin-resistant mutant of NCIMB8826	(4)
LM0187	NCIMB88261-R Δ*scrB*	This study
pRV300	pBluescript SK− with pAMβ1 Ermr gene; Amp^r, Erm^r	(38)
pRVscrB	pRV300 containing 1367-bp *L. plantarum* DNA between the SalI and SacI sites; Amp^r, Erm^r	This study

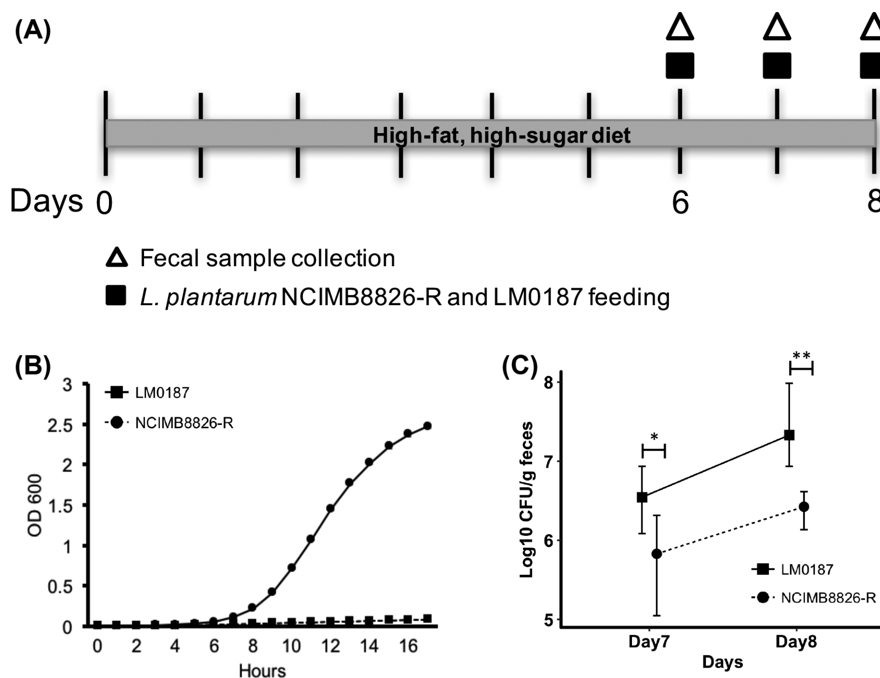

Figure 1. Increased *in vivo* fitness of *L. plantarum* LM0187. (A) Experimental design of the mouse study. (B) Growth of *L. plantarum* NCIMB8826-R and Δ*scrB* mutant LM0187 in modified MRS with 2% w/v sucrose as the carbon source. OD_{600} was measured every hour for a total of 16 h. (C) Culturable *L. plantarum* NCIMB8826-R and LM0187 in mouse stools 24 h after feeding as determined by colony enumeration. Points and whiskers indicate median, 1st and 3rd quartile of the corresponding treatment. $^{*}P<0.05$, $^{**}P<0.01$, Mann–Whitney U test.

(Tachon, Lee and Marco 2014). Fecal samples collected on day 8 were also flash frozen in liquid nitrogen and stored at −80°C until further analysis.

16S rRNA gene sequencing and data analysis

Total bacterial DNA was extracted from frozen stool samples collected on day 8, and the V4 regions of 16S rRNA genes were amplified by PCR as previously described (Yin *et al.* 2014). Pooled PCR amplicons were sequenced according to the paired-end protocol on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the UC Davis Genome Center (http://dnatech.genomecenter.ucdavis.edu/). Sequence data are available on Qiita (https://qiita.ucsd.edu, study number 10904) and deposited at the European Bioinformatics Institute (EBI) with accession number ERP022247.

Raw fastq files from both ends were assembled, demultiplexed and analyzed in the QIIME 1.8.0 (Quantitative Insights Into Microbial Ecology) software package (Caporaso *et al.* 2010). An average of 28,680 high-quality reads with a Phred score over 3 were obtained for each sample and 579 Operational Taxonomic Units (OTUs) with the abundance over 0.005% of the total reads were identified with 97% similarity using an open reference OTU picking method. Representative sequences from each OTU were assigned to their corresponding taxonomy according to the GreenGenes database (version 13_8) (DeSantis *et al.* 2006). Species identification was achieved using SPINGO with default parameters (Allard *et al.* 2015). Beta diversity was calculated using the UniFrac distance between samples (Segata *et al.* 2011) and visualized based on the results of principal coordinate analysis (PCoA). Bacterial gene contents were predicted through Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, version 1.0.0) (Langille *et al.* 2013). A total of 6,909 KEGG orthologs (KOs) were generated and after removing low abundant KOs (less than 100 counts), 2,895 KOs were obtained for further analysis. Discriminant KOs between different treatment groups were identified by LEfSe analysis with absolute value of Log LDA score over 2 (Segata *et al.* 2011).

Lactobacillus species were further identified by comparing the OTU DNA sequences against the NCBI 16S rRNA gene database (http://blast.ncbi.nlm.nih.gov). Due to the high sequence similarity of 16S rRNA gene V4 regions among *Lactobacillus* species, OTUs were classified to species clusters composed of *Lactobacillus* with identical V4 regions. Specifically, the *Lactobacillus murinus* cluster includes *L. murinus*, *Lactobacillus faecis*, *Lactobacillus apodemi* and *Lactobacillus animalis*. The *Lactobacillus plantarum* cluster includes *L. plantarum*, *Lactobacillus fuchuensis*, *Lactobacillus*

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composti, Lactobacillus graminis, Lactobacillus fabifermentans, Lactobacillus paraplantarum and *Lactobacillus pentosus*.

Lactobacillus species-specific, quantitative PCR

The numbers of *Lactobacillus* in the stools were measured by quantitative PCR (qPCR) using standard curves containing known genomic DNA copy numbers. To construct the standard curves, genomic DNA from *L. plantarum* NCIMB8826-R and *L. murinus* ASF 361 was extracted using the DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA) and quantified with the Quant-iT PicoGreen® dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA). A total of five 16S rRNA genes for each *L. plantarum* genome (Kleerebezem *et al.* 2003) and six 16S rRNA genes for each *L. murinus* genome (Sarma-Rupavtarm *et al.* 2004) were used to convert between corresponding cell numbers and 16S rRNA gene copy numbers. The DNA was then serially diluted to range between 10 and 10^7 16S rRNA gene copies per reaction and used for qPCR.

Primers K/L and M/N were used for *L. plantarum* and *L. murinus* quantification, respectively (Table S1, Supporting Information). The K/L primers were also predicted to target *L. pentosus* and M/N primers to target *L. murinus, L. animalis* and *L. crispatus* according to Primer-BLAST (Ye *et al.* 2012). Real-time, qPCR was performed in an ABI 7500 Fast Real-time PCR system (Applied Biosystems, Carlsbad, CA, USA). Each reaction contained SsoFast EvaGreen supermix with low ROX (2X) (Bio-Rad, Hercules, CA, USA), 250 nM of each primer (Life Technologies, Carlsbad, CA, USA), and approximately 30 ng of fecal bacterial genomic DNA or purified, diluted genomic DNA from *L. plantarum* NCIMB8826-R or *L. murinus* ASF 361. PCR amplification was initiated at 98°C for 3 min, followed by 40 cycles of 98°C for 5 s and 60°C for 30 s. Each reaction was performed in duplicate. A melting curve was added at the final stage to confirm the amplification specificity. PCR amplification efficiency was calculated each time based on the standard curve and data were only considered to be valid when the amplification efficiency was within 90%to 100%.

Metabolome analysis

There were a sufficient number of fecal samples to perform metabolomics on 33 mice (13, 8 and 12 mice from PBS, NCIMB88260R and LM0187 group, respectively). Samples were homogenized in cold deionized water, freeze-dried and dissolved in 10 mM phosphate buffer (pH 6.85). Wet and dry fecal weight were recorded. Samples were centrifuged at 6,000 g for 15 min followed by 14,000 g for 10 min to remove fecal particles and the supernatant was subsequently filtered through Amicon 3,000 molecular weight cutoff filters to remove protein and lipid particles. The filtrates were diluted with deionized water to a total volume of 207 μL and 23 μL of an internal standard (Chenomx Inc., Edmonton, Alberta, Canada) containing 4.86mM 3-(trimethylsilyl)-1-propanesulfonic acid-d 6 (DSS-d6) and 0.2% NaN3 in 98% D2O was added. The pH value was adjusted to 6.80± 0.12 for each sample by adding small quantities of 1 N HCl or NaOH. A total volume of 180 μL was transferred into a 3 mm NMR tube and stored at 4°C for further NMR analysis.

NMR spectra were acquired using the Bruker noesypr1d experiment on a Bruker Avance 600 MHz NMR spectrometer equipped with a SampleJet as previously described (Chen *et al.* 2014). Identification and quantification of metabolites were accomplished using Chenomx NMRSuite 7.6 (Chenomx Inc., Edmonton, Canada). Samples were corrected for dilution by multiplying by a correction factor calculated by the ratio of the final sample volume divided by the initial dry fecal weight. Metabolites found in more than 50% of the samples were used for further analysis. One sample in LM0187-fed group was identified to be the outlier through principal component analysis and was removed from further analysis (Filzmoser, Maronna and Werner 2008) (Fig. S1, Supporting Information). Both Spearman and Kendall rank correlation were performed followed by false discovery rate (FDR) correction between the metabolomic data and Log10 transformed CFU numbers of either *L. plantarum* NCIMB8826-R or LM0187 enumerated from the mouse stools.

Statistical analysis

Statistical analyses and plots were generated in Graphpad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) and R studio (Version 0.98.1091, RStudio, Inc., Boston, MA, USA). Unless specified, non-parametric statistical comparisons were performed, including Mann–Whitney U test and Kruskal–Wallis one-way analysis of variance with Dunn's post hoc tests. Multiple comparisons were corrected using FDR (Yoav and Yosef 1995).

RESULTS

Lactobacillus plantarum scrB is required for growth on sucrose

It was previously hypothesized that *L. plantarum* requires beta-fructofuranosidase encoded by *scrB* to grow on sucrose (Saulnier *et al.* 2007). This enzyme is responsible for the hydrolysis of sucrose-6-P into fructose and glucose-6-P. We investigated this possibility by constructing strain LM0187, a non-polar *scrB* deletion mutant of *L. plantarum* NCIMB8826-R. Unlike wild-type *L. plantarum* NCIMB8826-R, LM0187 could not grow in MRS when sucrose was provided as the sole carbon source (Fig. 1B). When glucose was provided, there were no significant differences between LM0187 and NCIMB8826-R growth rates and cell yields (Fig. S2, Supporting Information). The capacity to consume sucrose did not result in changes to osmotolerance because both strains grew equally in the presence of either ionic (1.4 M sodium chloride) or non-ionic (2.5 M sorbitol) compounds in MRS containing either glucose or glucose and sucrose (data not shown).

Lactobacillus plantarum LM0187 survived better than NCIMB8826-R in the murine GI tract

After acclimation to a HFHSD for 6 days, Balb/c mice were fed *L. plantarum* NCIMB8826-R or LM0187 for 2 consecutive days and the numbers of *L. plantarum* in the mouse stools were enumerated 24 h after each feeding (Fig. 1C, Fig. S3A & B). Although both strains survived GI tract passage according to viable cell enumerations on rifampicin-containing MRS, LM0187 was recovered in significantly higher numbers (mean ± se: 7.00 ± 0.20 Log_{10} CFU/ g feces) compared to NCIMB8826-R (6.10 ± 0.16 Log_{10} CFU/ g feces) (Fig. 1C). No rifampicin-resistant bacteria were detected in the sham, vehicle (PBS)-fed mouse stools (data not shown). *L. plantarum* species-specific, real-time quantitative (qPCR) also confirmed that there were higher numbers of the *scrB* mutant (2.31 ± 0.20 Log_{10} cell numbers/ ng DNA) in the mouse stools compared to the wild-type strain (1.57 ± 0.27 Log_{10} cell numbers/ ng DNA) (Fig. S3C, Supporting Information).

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Lactobacillus plantarum LM0187 and NCIMB8826-R differentially affected the fecal microbiota composition

Phylogenetic Diversity whole tree analysis indicated that the fecal bacterial diversity was similar between NCIMB8826-R-, LM0187-, and sham-fed mice (Fig. S4A, Supporting Information). The number of observed species (Fig. S4B, Supporting Information) and Shannon index (Fig. S4C, Supporting Information) were also unaffected by *L. plantarum* administration. Similarly, PCoA of the weighted UniFrac metric (beta-diversity) showed only a limited separation between the different mouse groups (Fig. 2A). Permutational analysis of variance (PERMANOVA) (Anderson 2001) using weighted and unweighted UniFrac distance matrices did not reveal significant differences ($P = 0.66$ and 0.43, respectively). UniFrac distance was also calculated from the cumulative-sum scaling (CSS) normalized OTU table (Paulson *et al.* 2013) and no significant changes were observed after either NCIMB8826-R or LM0187 feeding as determined by PERMANOVA based on either weighted ($P = 0.08$) or unweighted ($P = 0.49$) UniFrac distance metrics. The only notable difference was that fecal samples from LM0187-fed mice exhibited the greatest between and within group variance (Fig. 2B).

Mice fed *L. plantarum* LM0187 also showed changes in proportions of several fecal bacterial taxa (Table 2). The stools of those mice contained higher proportions of *Fecalibacterium* and *Streptococcus* and lower proportions of *Bacteroidaceae* compared to the sham controls (Table 2). Compared to mice given NCIMB8826-R, they harbored reduced levels of the *Coprococcus* genus (Table 2). However, it is notable that none of these genera exceeded 1% of the total bacterial population present.

16S rRNA gene sequences identical to *L. plantarum* were elevated in mice fed either *L. plantarum* NCIMB8826-R or LM0187 compared to the sham controls (Table 2). As expected according to CFU (Fig. 1C) and qPCR (Fig. S3C, Supporting Information) results, the proportions of *L. plantarum* OTUs were significantly higher in mice fed LM0187 compared to NCIMB8826-R (Fig. S3D, Supporting Information). Among the other *Lactobacillus* species in the mouse stools, the *L. murinus* cluster was the most abundant, contributing 7.04% ± 2.12% (mean ± se) of the total bacterial proportions in the control mice. The proportions of *L. murinus* were reduced to 5.01% ± 1.21% (mean ± se) and 4.84% ± 0.82% in mice fed NCIMB8826-R and LM0187, respectively; however, these differences were not significant (Fig. S5A, Supporting Information). *L. murinus* species-specific qPCR confirmed this finding (Fig. S5B, Supporting Information).

Lactobacillus plantarum LM0187 resulted in enrichment of lactate dehydrogenase genes according to metagenome predictions

Because taxonomic assessments do not fully address the functional changes that could occur in the gut microbiome with *L. plantarum* consumption, metagenome predictions were also made using PICRUSt (Langille *et al.* 2013). Such comparisons indicated that LM0187-fed mice harbored higher quantities of genes coding for lactate dehydrogenase (K00016, Fig. 3A). These genes were predicted to be mainly from unclassified *Streptococcaceae* (OTU 716006) (29% of the enrichment), as opposed to only 5.7% from *L. plantarum*. By comparison, alpha-fucosidase genes (K01206) were predicted to be more enriched in vehicle-fed mice compared to mice fed either *L. plantarum* strain (Table S2, Supporting Information). Forty percent of those genes were estimated to originate from an OTU of *Clostridiales* (OTU 351309).

Mouse fecal metabolites were changed by *L. plantarum* LM0187 feeding

Out of a library of 340 metabolites that included amino acids and derivatives, peptides, bile acids, sugars, nucleic acids, nucleotides and derivatives, organic and fatty acids, vitamins and co-factors and others, a total of 48 metabolites were detected in the mouse stools (Table S3, Supporting Information). Although glucose was present in the fecal contents of all mice, sucrose was not detected in any of the samples, indicating that the disaccharide was completely digested.

Corresponding to the metagenome predictions, lactate concentrations were higher in the stools of mice given LM0187 compared to the controls, although the increase did not reach significance ($P = 0.10$) (Fig. 3B; Table S3, Supporting Information). Glycerol, glutamate, tryptophan and uridine levels were also elevated (Fig. 4D; Table S3, Supporting Information). Conversely, concentrations of acetate, propionate, taurine, uracil and cytidine were higher in the stools of mice fed *L. plantarum* NCIMB8826-R compared to the vehicle-fed (PBS) controls (Fig. 4A–C; Table S3, Supporting Information). Glycerol was the only metabolite that differed significantly between the fecal contents of NCIMB8826-R and LM0187-fed mice (Fig. 4D; Table S3, Supporting Information).

In order to identify metabolites associated with *L. plantarum* survival, the metabolomes of individual mice were correlated with the numbers of culturable *L. plantarum* recovered from the stools. Spearman correlation analysis showed that taurine ($r = -0.71$) and uracil ($r = -0.71$) were highly negatively associated with strain NCIMB8826-R levels (Table 3). A total of twelve metabolites including uracil, ethanol and numerous amino acids were negatively correlated with the quantities of viable LM0187 according to the same correlation method and uracil remained significantly negatively correlated with LM0187 abundance after FDR correction (Table 3). None of the metabolites were significantly positively correlated with *L. plantarum* cell numbers. Kendall correlation confirmed these trends, although the only significant correlation was to uracil and this relationship was no longer found after FDR correction (Table S4, Supporting Information).

DISCUSSION

We addressed the possibility that the survival of probiotic *L. plantarum* in the GI tract is influenced by its capacity to metabolize sucrose, a primary constituent of obesogenic diets. Contrary to our expectations, the *L. plantarum* Δ*scrB* mutant was recovered in higher numbers in mouse stools than the wild-type strain. This result strongly indicates that dietary sucrose is not required as an energy source for *L. plantarum* in the intestine. Instead, the study showed that *L. plantarum* NCIMB8826-R sucrose metabolism influences the behaviors of this organism in the GI tract, at least when a high-fat, obesogenic diet is being consumed. Changes in the levels of fecal bacterial taxa and metabolites suggest that the wild-type *L. plantarum* and Δ*scrB* strains interact with the intestinal microbiota in different ways.

Sucrose is hydrolyzed by sucrase in the small intestine and the resulting glucose and fructose monomers are then metabolized by the epithelium and intestinal microbiota. Although it was expected that much of the dietary sucrose would be absorbed in the small intestine, we hypothesized that the capacity to consume sucrose would provide *L. plantarum* with at least a transient source of energy during GI transit that could elevate the numbers of surviving *L. plantarum* cells in the mouse

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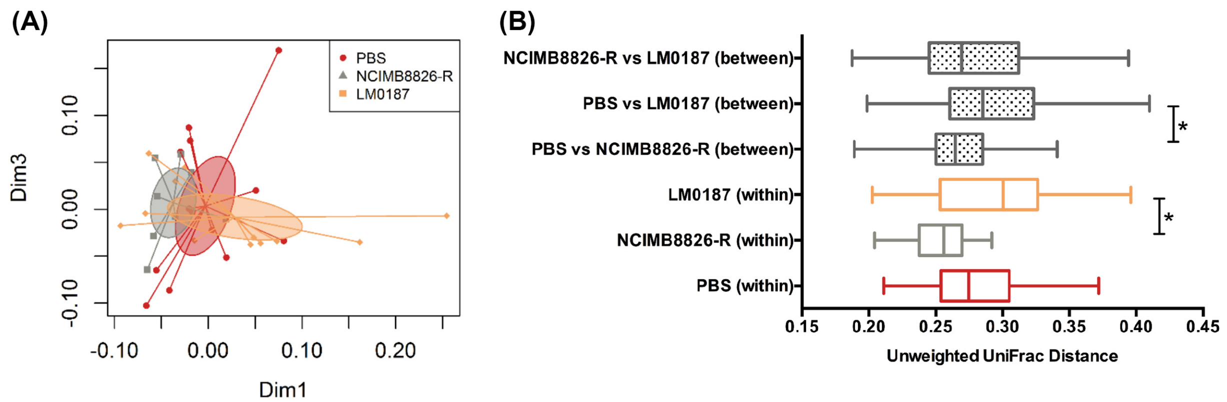

Figure 2. Mouse fecal microbiota composition was not significantly altered by *L. plantarum* NCIMB8826-R and LM0187 feeding. (A) Weighted UniFrac PCoA of the intestinal microbiota is shown. The ellipse encompasses the 95% confidence interval for each group. (B) Calculated unweighted UniFrac distances within and between groups are shown. To facilitate comparisons to the metabolome results, comparisons were limited to 8 mice fed *L. plantarum* NCIMB8826-R, 12 mice fed *L. plantarum* LM0187, and 13 PBS-administered sham controls. * $P < 0.05$ as determined by the permutation test (999 Monte Carlo permutations, Bonferroni-corrected).

Table 2. Bacterial taxa enriched in mouse fecal samples[a].

Comparison	Taxa	Relative abundance (median ± interquartile[b])		Log10 (LDA score[b])
		NCIMB8826-R	PBS	
NCIMB8826-R vs. PBS	*L. plantarum*	0.0045 ± 0.0068	0.0013 ± 0.0022	3.72
		LM0187	PBS	
LM0187 vs. PBS	*Bacteroidaceae*	0.0007 ± 0.0008	0.0015 ± 0.0007	3.53
	L. plantarum	0.0218 ± 0.0309	0.0013 ± 0.0022	4.30
	Faecalibacterium	0.0022 ± 0.0011	0.0011 ± 0.0006	3.68
	Streptococcus	0.0023 ± 0.0009	0.0014 ± 0.0008	3.75
		NCIMB8826-R	LM0187	
NCIMB8826-R vs. LM0187	*Coprococcus*	0.0004 ± 0.0002	0.0002 ± 0.0001	2.49

[a]Enriched taxa were identified by LEfSe (45).
[b]The 1st and 3rd quartiles are shown.
[c]Linear Discriminant Analysis score.

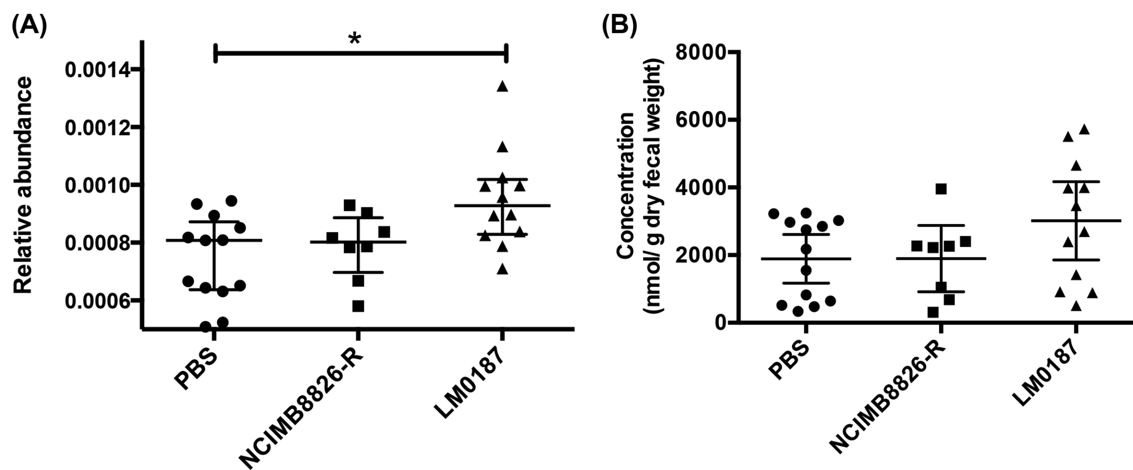

Figure 3. Increased lactate dehydrogenase activity in the fecal contents of mice fed LM0187. (A) Relative gene counts of K00016 as predicted through PICRUSt (14) and (B) lactate concentrations in mouse fecal contents. *$P < 0.05$, Kruskal–Wallisone-way analysis of variance with Dunn's post hoc tests.

feces. However, we observed the opposite result, and the wild-type strain NCIMB8826-R was recovered in significantly lower levels than the Δ*scrB* mutant according to viable *L. plantarum* cell counts in the stools measured one day after administration. This change was consistent with the qPCR and 16S rRNA gene amplicon sequence data. The differences in *L. plantarum* cell numbers are notably similar to those found when *L. plantarum* recovery was measured for mice fed LFPP and HFHS diets (Tachon, Lee and Marco 2014; Yin *et al.* 2017).

Although the benefits of sugar metabolism for microorganisms in the GI tract are well known (Chang *et al.* 2004; Iyer and Camilli 2007; Le Bouguénec and Schouler 2011; Pereira and Berry

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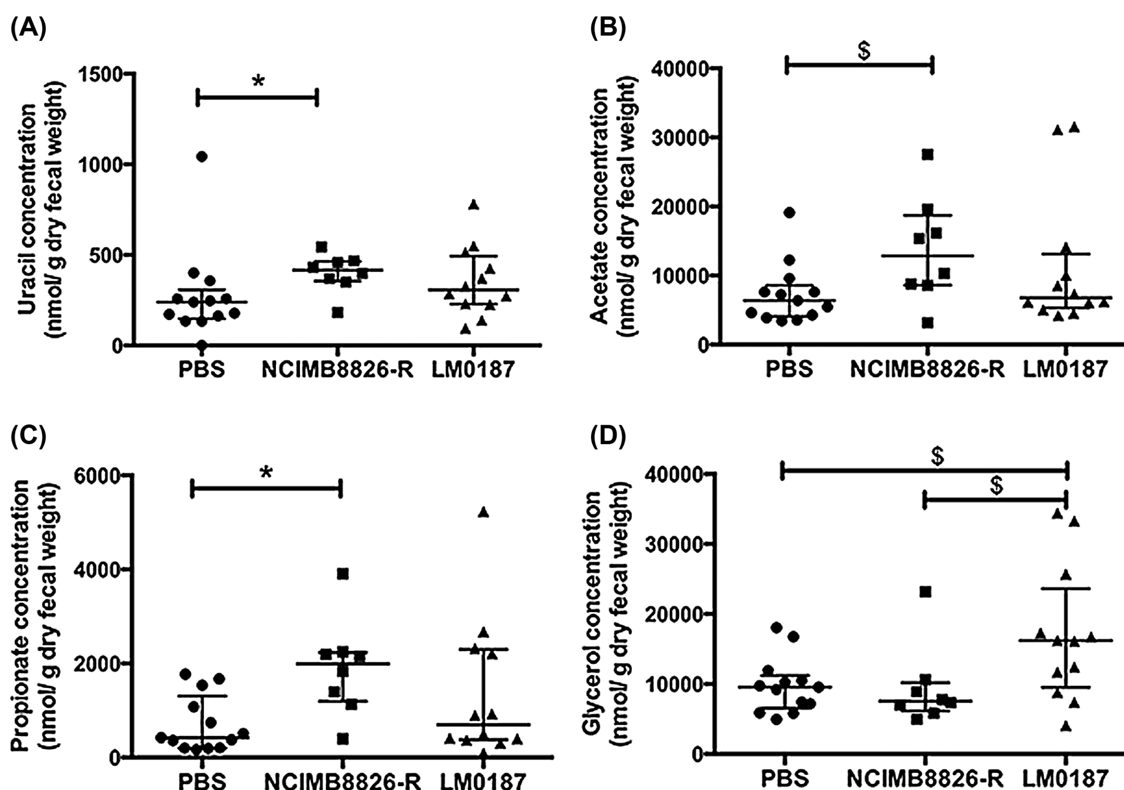

Figure 4. Metabolite concentrations significantly changed in mouse fecal contents. (A) uracil, (B) acetate, (C) propionate, and (D) glycerol are shown. *P <0.05, Kruskal–Wallis one-way analysis of variance with Dunn's post hoc tests. $ P <0.05, Mann–Whitney U test.

Table 3. Fecal metabolites significantly correlated with either *L. plantarum* NCIMB8826-R or LM0187 fecal numbers.

		L. plantarum NCIMB8826-R			*L. plantarum* LM0187		
Category	Metabolites	r[a]	P value	P value after FDR correction	r	P value	P value after FDR correction
Amino acids and associated metabolites	Taurine	**– 0.71**[b]	**0.05**	**0.87**	0.18	0.57	0.74
	Glutamate	0.38	0.35	0.87	**– 0.75**	**0.01**	**0.12**
	Asparagine	0.55	0.16	0.87	**– 0.64**	**0.02**	**0.16**
	Alanine	0.43	0.29	0.87	**– 0.63**	**0.03**	**0.17**
	Urocanate	0.48	0.23	0.87	**– 0.61**	**0.04**	**0.19**
	Methionine	0.33	0.42	0.87	**– 0.59**	**0.04**	**0.21**
	Lysine	0.14	0.74	0.87	**– 0.58**	**0.05**	**0.21**
	Tyrosine	0.29	0.49	0.87	**– 0.57**	**0.05**	**0.21**
	2-Oxoisocaproate	0.52	0.18	0.87	**– 0.68**	**0.02**	**0.15**
Nucleotides and derivatives	Uracil	**– 0.71**	**0.05**	**0.87**	**– 0.88**[c]	**0.00**	**0.01**
	Cytidine	– 0.14	0.74	0.87	**– 0.68**	**0.02**	**0.15**
	Hypoxanthine	– 0.12	0.78	0.87	**– 0.65**	**0.02**	**0.16**
Microbiota derived	Ethanol	– 0.29	0.49	0.87	**– 0.68**	**0.02**	**0.15**

[a]Spearman correlation coefficient is shown.
[b]Numbers in bold indicate significant correlations before FDR correction.
[c]Numbers in bold and underlined indicate significant correlations after FDR correction.

2017), it is not yet understood how the loss of this catabolic activity could alter *L. plantarum* transit times or recovery from the mouse intestine. One possibility is that the inability to consume sucrose as a source of carbon and energy resulted in increased access of that carbohydrate to indigenous bacterial species that could then provide a more hospitable environment to *L. plantarum* during passage through the gut. Alternatively, the loss of ScrB might have caused unforeseen metabolic or physiological consequences to *L. plantarum* in the intestine such as altering adherence of the probiotic to the epithelium or modifying host-microbe interactions to confer changes in intestinal motility. With regard to the latter, *L. plantarum* was previously shown to stabilize intestinal motility (Niedzielin, Kordecki and

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Birkenfeld 2001; Li *et al.* 2015) and this might occur in a strain-specific manner. The changes observed for the LM0187 strain could result in transit times that are longer or shorter than wild-type NCIMB8826-R, and hence not be distinguishable by the time points measured here. Lastly, even though we have shown that the mutant and wild-type strain performed equally well in utilizing glucose and tolerating osmotic stresses *in vitro*, a more thorough characterization of the ScrB deletion mutant and analysis of other proteins required for sucrose metabolism by *L. plantarum* are needed to fully investigate the scope at which this enzymatic pathway is relevant to *L. plantarum in vivo*.

Both *Faecalibacterium* and *Streptococcus* were enriched in the LM0187-fed mice. *Faecalibacterium* is a butyrate-producing member of the *Clostridiaceae* family that has the capacity for cross-feeding on organic acids (Rios-Covian *et al.* 2015). Increased levels of *Faecalibacterium* in human subjects were previously observed following probiotic *Lactobacillus* consumption (Larsen *et al.* 2011; Derrien and van Hylckama Vlieg 2015; Zhang *et al.* 2014). *Streptococcus* is a LAB that produces lactic acid as a primary metabolic end-product. Metagenome predictions indicated that the increased lactate dehydrogenase gene counts observed in the LM0187-fed mice could be mostly attributed to the *Streptococcus* genus. The enrichment of *Streptococcus* in *L. plantarum*-fed mice corresponds well with the “like will to like” rule observed for *Lactobacillus reuteri* and host indigenous lactobacilli (Stecher *et al.* 2010). It is also notable that increased proportions of *Streptococcus* were also observed in mice fed *L. plantarum* WCFS1 and the HFHSD in another study (Yin *et al.* 2017). Although it is not clear why the proportions of *Streptococcus* were only significantly higher in mice fed the mutant strain and not wild-type *L. plantarum*, numerous *Streptococcus* species can consume sucrose for growth and therefore might have had greater access to this carbohydrate than in the control mice or those fed NCIMB8826-R.

Although stools from mice consuming LM0187 contained higher concentrations of lactate, levels of acetate and propionate were greater in mice fed *L. plantarum* NCIMB8826-R compared to those fed either PBS or LM0187. These changes in short-chain fatty acids (SCFAs) strongly indicate the activation of different fermentative pathways is dependent on the carbohydrate metabolism capacities of the ingested probiotic strain. Increases in intestinal SCFA induced by probiotics might constitute a core benefit of probiotics in the GI tract because SCFA are increasingly associated with colonic health, host immune regulation, energy metabolism, host appetite as well as the regulation of the epigenome (Wong *et al.* 2006; Smith *et al.* 2013; Hill *et al.* 2014; Mischke and Plösch 2016; van de Wouw *et al.* 2017). It is notable that changes in metabolism of this one sugar could result in different concentrations of fermentation end-products in the intestine.

Mice consuming LM0187 harbored significantly higher quantities of glycerol. Intestinal glycerol can originate from the diet, microbial synthesis, or from desquamated epithelial cells (De Weirdt *et al.* 2010). Because certain gut microbes can quickly metabolize glycerol, fecal glycerol concentrations are usually low in healthy individuals (Marchesi *et al.* 2007). *L. plantarum* does not synthesize glycerol but rather assimilates it through the oxidative pathway mediated by glycerol kinase and glycerol-3-P dehydrogenase and fed into glycolysis (Rivaldi *et al.* 2013). Induction of glycerol metabolism was observed for *L. plantarum* in mono-associated mice (Marco *et al.* 2009). The increase in glycerol combined with the negative correlation between LM0187 proportions and fecal ethanol concentrations, further supports the possibility that general fermentative metabolism in the intestine was significantly altered in a manner that was dependent upon the capacity of *L. plantarum* to consume sucrose.

A total 12 metabolites were negatively correlated with *L. plantarum* LM0187 cell numbers. Most of them were amino acids and amino acid breakdown products, indicating the lack of *L. plantarum* ScrB resulted in other metabolic changes among the intestinal microbiota. For *L. plantarum* NCIMB8826-R-fed mice, both taurine and uracil were negatively correlated with cell numbers. Lumenal taurine is known to influence host physiology and the gut microbiota (Yu *et al.* 2016) and therefore might also impact *L. plantarum* GI tract transit. Although uracil concentrations were highest in mice fed *L. plantarum* NCIMB8826-R, there was an inverse correlation between *L. plantarum* quantities and uracil amounts. It was previously shown in *Drosophila* that intestinal uracil can serve as a ligand for dual oxidase (DUOX)-dependent reactive oxygen species (ROS) generation important for maintaining intestinal immune homeostasis (Lee *et al.* 2013, 2015c). Although the effect of uracil on ROS generation in mice and other mammals has yet to be shown, ROS are well recognized to alter gut microbiota composition and function (Neish *et al.* 2004) and therefore should be investigated for a potential role in regulating the cell numbers of *L. plantarum* and possibly other bacteria in the GI tract.

In conclusion, we found that the loss of sucrose metabolism by *L. plantarum* altered transit of this organism in the murine GI tract and this was associated with some distinct changes in the intestinal microbiota and metabolomes compared to mice fed wild-type strain NCIMB8826-R. Because lactobacilli, including strains of *L. plantarum*, are increasingly recognized to benefit human health (Panigrahi *et al.* 2017; Heeney, Gareau and Marco 2018), understanding how dietary components impact these bacteria is essential for optimizing approaches to increase the intestinal persistence and survival behaviors and interactions with the other microorganisms in the GI tract.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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