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Effect of potato fiber on survival of *Lactobacillus* species at simulated gastric conditions and composition of the gut microbiota in vitro

Nadja Larsen^{a,*}, Carlota Bussolo de Souza^b, Lukasz Krych^a, Witold Kot^c, Thomas Dyrmann Leser^d, Ole Bandsholm Sørensen^e, Andreas Blennow^f, Koen Venema^b, Lene Jespersen^a

^a Department of Food Science, University of Copenhagen, Frederiksberg C 1958, Denmark

^b Center for Healthy Eating and Food Innovation, Maastricht University – campus Venlo, the Netherlands

^c Department of Environmental Science, Aarhus University, Roskilde, Denmark

^d Chr. Hansen A/S, Hoersholm, Denmark

^e KMC, Brande, Denmark

^f Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C 1958, Denmark

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ABSTRACT

Potato fiber is a side product in starch manufacturing rich in dietary fibers such as pectin, cellulose, hemicellulose and resistant starch. So far, the beneficial properties of potato fiber have been poorly characterized. This study investigated the effect of FiberBind 400, a commercial potato fiber product, on survival of probiotic *Lactobacillus* strains at simulated gastric conditions and on the composition and metabolic activity of the gut microbiota, using the TIM-2 colon model. Resistant starch and native starch from potato were used as reference substrates. FiberBind 400 had an ability to improve survival of the four tested strains, *Lactobacillus fermentum* PCC[®], *L. rhamnosus* LGG[®], *L. reuteri* RC-14[®] and *L. paracasei* F-19[®] in a strain-dependent way. The highest effect was observed for *L. fermentum* PCC[®] and *L. rhamnosus* LGG[®]. The effect of starches on bacterial survival was insignificant. Composition of the fecal microbiota in TIM-2 fermentations was assessed by high-throughput sequencing of 16S rRNA gene amplicon. Fermentation of FiberBind 400 resulted in more diverse microbial communities compared to starches. Changes in microbial abundances specifically mediated by FiberBind 400, included increases in the genera *Lachnospira*, *Butyrivibrio*, *Mogibacterium*, *Parabacteroides*, *Prevotella* and *Desulfovibrio*, and the species *B. ovatus*, as well as decreases in *Ruminococcus torques* and unassigned *Ruminococcus* spp. Shifts in other bacterial populations, such as increased abundances of *Oscillospira*, *Enterococcus*, *Bacteroidales*, *Citrobacter*, along with reduction of *Roseburia*, *Ruminococcus*, and *Faecalibacterium prausnitzii* were not significantly different between the substrates. Cumulative production of individual short-chain fatty acids was similar between potato fiber and starches. The study demonstrated that FiberBind 400 had a potential to protect probiotic *Lactobacillus* strains during the passage through the gastrointestinal tract and selectively modulate the gut bacterial populations. This knowledge can support application of potato fiber as a functional food ingredient with added biological benefits.

1. Introduction

Dietary fibers (DF) are typically plant polysaccharides indigestible by enzymes in the human intestine. Reaching the colon, DF can be degraded and utilized by the gut bacteria with production of short-chain fatty acids (SCFA), gases and other metabolites (Lockyer & Nugent, 2017). The types of dietary fibers, which can be selectively utilized by host microorganisms, conferring a health benefit are claimed as prebiotics (Gibson et al., 2017). The effects of DF on the gut health are mediated through the compositional changes of the gut microbiota

and production of microbial metabolites, and depend to a large extent on the fiber type (Wang et al., 2019). One of the potential candidates of prebiotic fibers is potato fiber, a side product of starch manufacturing rich in starch, pectin, cellulose and hemicellulose (Lovegrove et al., 2017; Pastuszewska, Antushevich, Tuśnio, & Taciak, 2009; Zhao, Andersson, & Andersson, 2018). The yield of potato fiber is about 14 kg per ton of starch potatoes, and only in Europe potato fiber is currently produced in millions of tons per year (Grommers & van der Krogt, 2009; Starch EU, 2018). In addition to a rapidly digestible starch, potato fiber contains resistant starch (RS), which nutritional properties, digestibility

* Corresponding author at: Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark.

E-mail address: nf@food.ku.dk (N. Larsen).

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and beneficial effects in the gut have been extensively characterized (Yang et al., 2017; Zhang et al., 2015). Human intervention studies demonstrated that dietary supplementation with resistant starch improved glycemic status and inflammatory markers in patients with type 2 diabetes and metabolic syndrome (Johnston, Thomas, Bell, Frost, & Robertson, 2010; Karimi et al., 2016). It has been suggested that replacing digestible dietary starches with resistant starch would contribute to weight management due to the lower calorie content and energy density (Zhang et al., 2015). The prebiotic potential of pectins, another component of potato fiber, was also confirmed by several in vitro and in vivo studies, showing the ability of pectins to modulate specific populations of fecal microbiota, to induce gut immunity, decrease energy intake and improve intestinal integrity (Fukunaga et al., 2003; Gómez, Gullón, Yáñez, Schols, & Alonso, 2016; Larsen et al., 2019; Salleh, Fairus, Zahary, Bhaskar Raj, & Mhd Jalil, 2019). Furthermore, both resistant starch and pectins can be beneficially used for microencapsulation of *Lactobacillus* spp. and *Bifidobacterium* spp., to increase viability of bacterial cells during the gastro-intestinal transit (Etchepare et al., 2016; Zanjani, Ehsani, Tarzi, & Sharifan, 2018). Recently, we demonstrated that the ability of pectins to protect probiotic *Lactobacillus* strains exposed to intestinal stresses was influenced by the structural and physical properties, among them, degrees of methylation and branching, surface charges, molecular weight and viscosity (Larsen, Cahú, Saad, Blennow, & Jespersen, 2018). Though extensive data exists on the individual components, the role of potato fiber as a whole is investigated insufficiently. A few published studies indicated that dietary potato fiber given to rodents stimulated caecal fermentation, improved mineral absorption (Pastuszewska, Taciak, Tuśnio, & Misztal, 2010) and attenuated inflammation during induced colitis in rats, potentially through SCFA production (Panasevich et al., 2015). A combination of potato fiber and red meat diets in rats balanced the harmful impact of red meat by increasing colonic *Bifidobacterium* spp. and improving biomarkers of bowel health (Paturi et al., 2012).

The growing evidence of beneficial impact of dietary fibers encourages a search for the new fiber-based products. This study focused on commercial potato fiber produced under the brand name FiberBind 400 (KMC, Denmark). The product has a high water-binding capacity and can be applied as a functional ingredient (stabilizer) in such food products as sausages and cold cuts. This in vitro study investigated the prebiotic potential of FiberBind 400, including its ability to increase survival of probiotic *Lactobacillus* strains at gastro-intestinal conditions and to modulate the gut microbiota. The reference substrates, native starch and resistant starches from potato, were included in the experiments for data comparison. The microbiota fermentations were performed in TIM-2 model of proximal colon, developed by The Netherlands Organization for Applied Scientific Research (TNO).

2. Methods

2.1. Potato fiber and starches

FiberBind 400 and starches were commercial products from a potato variety *Solanum tuberosum* produced at KMC (Brandø, Denmark). According to the KMC certificate (unpublished data), FiberBind 400 contained per 100 g: 65 g dietary fiber (pectin, cellulose and hemicellulose), 20 g starch, 5 g protein, 2 g fat and 0.5 g sodium chloride. The starch fraction was composed of digestible starch and resistant starch fractions, accounting for 12% and 8% of FiberBind 400, respectively. Reference substrates in this study were native potato starch (NS) and cross-linked resistant starch from potato (RS). Reagents for the experiments were purchased from Merck A/S (Hellerup, Denmark) and Sigma-Aldrich Aps (Broendby, Denmark) unless otherwise stated.

2.2. High Performance Anion Exchange Chromatography

Composition of monosaccharides in FiberBind 400 was determined

by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) as reported previously (Larsen et al., 2018). Shortly, suspensions of FiberBind 400 in water were hydrolysed in trifluoroacetic acid and injected into PA20 column (Dionex, CA, USA). Before injection the column was washed and equilibrated with NaOH. The elution program included an isocratic elution with water, followed by a linear gradient elution with NaOH (up to 1 M). Calibration was performed with a standard solution containing galacturonic acid, arabinose, rhamnose, galactose, glucose and xylose. The HPAEC-PAD analyses were performed in duplicates.

2.3. Bacterial strains and growth conditions

Commercial probiotics *Lactobacillus fermentum* PCC®, *Lactobacillus reuteri* RC-14®, *Lactobacillus paracasei* subsp. *paracasei* F-19® and *Lactobacillus rhamnosus* LGG® were provided by Chr. Hansen A/S (Hoersholm, Denmark). Strains were maintained in de Man Rogosa Sharpe (MRS) broth (Becton Dickinson A/S, Albertslund, Denmark) with 20% (v/v) glycerol at −80 °C. Before the experiments bacterial strains were plated onto MRS agar and incubated anaerobically (AnaeroGen sachets; Oxoid A/S, Roskilde, Denmark) at 37 °C for 48 h.

2.4. Test of bacterial survival at simulated gastric conditions

Bacterial survival was tested in the simulated gastric solution (GS) which contained 0.31 g sodium chloride, 0.11 g potassium chloride, 0.06 g sodium bicarbonate, 0.06 g calcium chloride dehydrate, 8 U/ml lipase (from porcine pancreas) and 120 U/ml pepsin (from porcine gastric mucosa) in a total volume of 100 ml citrate/phosphate buffer (final concentration 0.1 M) (Barker, Abrahamsson, & Kruusmägi, 2014). The pH was adjusted to 2.5 (± 0.01) with 0.1 M HCl. Potato fiber, native starch or resistant starch were added to GS (1% (w/v)) for test experiments and MilliQ water was added for controls. Bacterial strains were inoculated in MRS broth from a single colony on the MRS agar plates and grown overnight anaerobically at 37 °C. Cells from overnight cultures were re-suspended in saline solution (0.85% (w/v) NaCl) and added to the GS test and control solutions to a final cell density of 10⁹ CFU/ml. Survival kinetics of the strains was followed during incubation at 37 °C by flow cytometry and standard plating on MRS agar. Experiments were performed in triplicates.

2.5. Bacterial viability by flow cytometric analysis and plate counting

To determine the changes in live cells fractions during incubation in GS, bacterial cells were collected by centrifugation, washed in saline solution and stained with fluorescent markers SYTO®13 (Life Technologies Europe BV, Denmark) and propidium iodide (PI). Negative controls (dead cells), were prepared by treatment of cell suspensions with 70% ethanol before staining. Emission was recorded at 530 ± 20 nm from SYTO®13 stained cells (green fluorescence), and at 692 ± 20 nm from PI stained cells (red fluorescence) using BD FACS Jazz Cell Sorter (BD Biosciences, USA). Subpopulations of live and dead cells were estimated from the dot plot images (cytograms) based on 100,000 events per sample using the FlowJo software (version 10, Tree Star Inc. USA). The colony forming units (CFU) were determined by plating serial dilutions of cell suspensions onto MRS agar after 4 h treatment of strains *L. fermentum* PCC® and *L. reuteri* RC-14®, and after 1 h treatment of strains *L. rhamnosus* LGG® and *L. paracasei* F-19® to obtain at least 1 Log₁₀ CFU reduction. The MRS agar plates were incubated anaerobically at 37 °C for 48 h. Differences in Log₁₀ CFU reduction between the treatments and control experiments were evaluated by the paired two-tailed Student's *t*-test (Microsoft Office Excel 2010).

2.6. TIM-2 fermentations and sample collection

The TIM-2 colon model, including operational units, conditions of fermentation and sample loading has been described previously (Venema, 2015). Inoculum consisted of pooled fecal microbiota collected from 8 healthy Caucasian adults (male: $n = 4$, female: $n = 4$; age of 25–42 years). TIM-2 fermentations were performed with 30 ml portion of microbiota at 37 °C and pH 5.8. Before treatments the microbiota was incubated for 16 h for adaptation to the new environment. During the adaptation period the basal simulated ileal efflux medium (SIEM) was gradually introduced in a total volume of 40 ml per unit; afterwards, the microbiota was deprived from any medium for 2 h (starvation period). The units were then fed with SIEM containing 7.5 g of FiberBind 400, native starch or resistant starch per day as the only carbohydrate source. Luminal and dialysate samples were collected at fermentation start (0 h) and after 24, 48, 56 and 72 h for analyses of microbiota composition, short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA). Fermentations of each substrate were performed as two independent TIM-2 experiments.

2.7. DNA purification and sequencing

The total bacterial DNA was purified using the PowerLyzer® PowerSoil DNA Isolation Kit according to the manufacturer's protocol (Qiagen Nordic, Denmark). Before the bead beating step, fecal slurries were resuspended in the Bead Solution and incubated at 65 °C for 10 min. The homogenization was performed in three cycles, 30 s each with 30 s interval, at 6.5 m/s (FastPrep®-24, MP Biomedicals, Solon OH, USA). The DNA concentration was commonly between 20 and 100 ng/μl and A260/A280 ratios were of 1.8–2.1 (NanoDrop™ 2000 Spectrophotometer, ThermoFisher Scientific, Denmark). The DNA library was prepared as described previously (Larsen et al., 2019). In brief, the V3 region (~190 bp) of the 16S rRNA gene was PCR amplified using AccuPrime SuperMix II (Life Technologies, CA, United States) and primers comparable with Nextera Index Kit, NXt_388F and NXt_518R (Illumina). To incorporate adapters and tags, the second PCR step was carried out using Phusion High-Fidelity PCR Master Mix (ThermoFisher Scientific, MA, United States) and primers P5 and P7 (Nextera Index Kit). Sequencing was conducted using 2 × 150 cycles, MID output and V2 chemistry on the Illumina NextSeq 500 platform (Illumina, CA, USA). The raw dataset containing pair-ended reads were merged, trimmed and clustered (operational taxonomic units, OTUs, with 97% similarity) using the UPARSE pipeline.

2.8. Analysis of sequencing data and statistic

Sequencing data were analyzed using QIIME software (version 1.8.0) (Caporaso et al., 2010) and the Greengenes database (version 13.8) for taxonomy annotation (McDonald et al., 2012). Alpha-diversity was evaluated by the number of observed species and Chao1 (97% similarity OTUs) computed for each OTU table rarefied to 10,000 sequences per sample, based on the lowest number of sequences produced per sample. Differences in alpha-diversity between the substrates were tested using Student's *t*-test, employing the non-parametric (Monte Carlo) method (999 permutations). Community differences (beta-diversity) were revealed using weighted and unweighted UniFrac distances matrices and evaluated by analysis of similarities (ANOSIM). A weighted UniFrac matrix is based on the absolute proportions, and it is most sensitive to distinguish abundant bacterial populations, while an unweighted matrix relies on the presence or absence of bacteria, accounting for both dominant and rare taxa. Data matrix was built with sample number ($n = 30$, columns) versus species number (1059 OTU, rows). Principal Coordinate Analysis (PCoA) plots were generated with Jackknifed Beta Diversity workflow using 10 subsampled OTU tables. Bacterial abundances in the pooled data set (48, 56 and 72 h) obtained in two independent experiments ($n = 6$ for each substrate) were

compared to fermentation start (0 h, $n = 6$). Statistical differences were assessed by the nonparametric Wilcoxon Rank Sum test combined with Bonferroni multiplicity correction, using 0.05 as significance level.

2.9. Analysis of short-chain and branched-chain fatty acids

Cumulative production of SCFA and BCFA was quantified in the lumen and dialysate TIM-2 samples by Brightlabs (Venlo, the Netherlands). Supernatants were diluted with 1.5 mM sulfuric acid and injected onto an 883 Basic IC plus ion-chromatography system (Metrohm, Herisa, Switzerland). The acids were eluted at a flow rate of 0.4 ml/min with a pressure of 5.5 MPa at 65 °C using ICsep ION300 Ion exclusion column and Metrosep RP2 Guard column (Transgenomic, New Haven, CT, USA). Acetic, propionic, butyric, isobutyric and isovaleric acids were used as calibration standards (Sigma Chemical, St. Louis, MO). Differences in production of SCFA and BCFA were evaluated by the paired two-tailed Student's *t*-test (significance level $p < .05$).

3. Results

3.1. Composition of potato fiber FiberBind 400

The major components of potato fiber included dietary fiber, i.e., pectin, cellulose and hemicellulose, and starch (KMC certificate, unpublished data). Neutral sugar analysis of FiberBind 400 showed that 48.0% of total monosaccharides was glucose, originating primarily from the starch, cellulose and pectin components. The other dominant sugar residues were galactose 14.8%, arabinose 11.8% and xylose 9.6%, the common constituents of pectin and hemicellulose molecules (Chan, Choo, Young, & Loh, 2017). In addition, pectin in FiberBind 400 was a source of galacturonic acid (13.1%) and rhamnose in low amounts (0.5%).

3.2. Survival of *Lactobacillus* spp. in gastric solution as affected by potato fiber

Viability of the tested *Lactobacillus* strains in gastric solution was strain-specific and significantly improved in the presence of FiberBind 400 compared to control experiments (GS without added carbohydrates) as determined by flow cytometry (Fig. 1). The highest increase in live cell fractions was observed for *L. fermentum* PCC® and *L. rhamnosus* LGG® throughout the whole incubation period (Fig. 1A and C). Thus, the live cells of *L. fermentum* PCC® comprised $45.9 \pm 5.5\%$ after 6 h treatment with potato fiber compared to $4.8 \pm 2.2\%$ in controls; while the live cells of *L. rhamnosus* LGG® were increased to $18.8 \pm 6.1\%$ at the end of test incubations (2 h) versus $0.8 \pm 1.6\%$ in controls. The live cells percentage of *L. reuteri* RC-14® and *L. paracasei* F-19® was increased by potato fiber mainly in the first part of incubation (Fig. 1B and D). The flow cytometry results were confirmed by CFU plating, showing an increase of viable counts of *Lactobacillus* spp. in gastric solution in the presence of FiberBind 400 compared to control incubations (Fig. S1, Supplementary Material). The effect of starches on bacterial resistance to gastric solution was not statistically different from controls (Figs. 1 and S1).

3.3. Alpha-diversity of microbiota

Alpha-diversity of microbiota over time is presented by Chao1 indices (Fig. 2) and rarefaction curves computed for the observed species number (Fig. S2, Supplementary Material). Fermentations of FiberBind 400 resulted in significant increase of alpha-diversity levels between 24 and 56 h (Chao1 of 747–758). In starch fermentations the increase in diversity was recorded after 24 h (Chao1 of 738 ± 29 for resistant starch and 773 ± 40 for native starch), followed by gradual decrease, lowest in native starch fermentations (Chao1 of 631 ± 37 after 72 h).

Fig. 1. Survival of probiotic *Lactobacillus* strains (A) *L. fermentum* PCC, (B) *L. reuteri* RC-14, (C) *L. rhamnosus* LGG and (D) *L. paracasei* F-19 in simulated gastric solution (GS) with 1% (w/v) of FiberBind 400 (▲, dashed line) or resistant starch (●, solid line), and controls (Δ, dots). Live cells fractions (%) were determined by flow cytometry using SYTO®13 and propidium iodide staining and presented by mean values and SD (bars) from triplicate experiments. Significant differences between FiberBind 400 and controls are denoted by asterisks (Student's *t*-test, two-tailed, **p* < .05; ***p* < .01).

FiberBind 400 produced more diverse microbial communities (*p* < .05) between 48 and 72 h fermentation compared to starches.

3.4. Beta-diversity of microbiota as affected by substrates and sampling time

Analysis of bacterial beta-diversity (or group diversity) showed clear separation between the fermentation substrates, i.e., potato fiber, resistant starch and native starch, as illustrated by the PCoA plots (Fig. 3A). The group separation was best explained with the use of weighted UniFrac distance matrix (principal components PC1 56% and PC2 23%) which consider mostly changes in abundant lineages,

indicating that proportions of dominant rather than rare bacterial taxa contributed to the variance between the groups. The PCoA plots build up across the sampling times (Fig. 3B) showed that the major changes in microbiota occurred after the first 24 h fermentation (*p* = .01). Differences between the time-points 48, 56 and 72 h were insignificant (*p* = .92) suggesting stabilization of microbial community structure.

3.5. The effect of potato fiber and starches on microbiota composition

Sequencing analysis of 16S rRNA gene amplicons generated in total 110 OTUs at species-level. Based on results of the beta-diversity analysis, showing similarity in microbiota after > 48 h fermentation (Fig. 3B), statistical differences were evaluated for the combined data (48, 56 and 72 h) obtained for each substrate. Bacterial taxa which relative abundances were significantly affected by FiberBind 400 and the corresponding changes observed in fermentations of starches are presented in Fig. 4. Bacterial populations stimulated highest by FiberBind 400 were assigned to the order *Clostridiales*, the genera *Lachnospira*, *Butyrivibrio* and *Mogibacterium*, belonging to the phylum *Firmicutes*, the genera *Prevotella* and the species *Bacteroides ovatus*, belonging to the phylum *Bacteroidetes*, and to the members of the family *Desulfovibrionaceae* (*Desulfovibrio* D168) within the phylum *Proteobacteria*. Bacterial taxa increased to lesser extent by potato fiber, compared to resistant starch and/or native starch, included the genera *Clostridium*, *Bacteroides* (*B. uniformis*), *Parabacteroides* (*P. distasonis*), and the families *Ruminococcaceae*, *Christensenellaceae*, *Clostridiaceae* and *Mogibacteriaceae*. Growth of *Bifidobacterium* (phylum *Actinobacteria*) was most extensively promoted by native starch. The family *Lachnospiraceae* and the species *Prevotella copri*, prevalent at fermentation start (21.3% and 15.8%, respectively), were decreased by the treatments, highest by native starch. A few bacterial taxa, such as *Ruminococcus* (proposed taxonomy), *R. torques* and *Paraprevotella*, were found in lower abundances in potato fiber fermentations. Changes in other commensals, including increased abundances of *Oscillospira*, *Enterococcus*, *Bacteroidales*, the order RF39 (phylum *Tenericutes*), and *Proteobacteria* (*Citrobacter* and *Bilophila*), along with a reduction in *Roseburia*, *Ruminococcus*, *Faecalibacterium prausnitzii* and

Fig. 2. Alpha-diversity of microbiota during fermentation of FiberBind 400 (FB), resistant starch (RS) and native starch (NS) expressed by Chao1 index. Chao1 values at baseline (0h, grey columns), 24 h (green columns), 48 h (yellow columns) and 72 h (blue columns) fermentation are presented by means and standard deviation (bars) from two independent TIM-2 experiments. Microbiota was analyzed using Illumina NextSeq 500 Sequencing System. Asterisks denote the values significantly different from time 0h (two-tailed Student's *t*-test, **p* < .05, ***p* < .01). Significant differences between FiberBind 400 and starches computed for combined data set (48, 56 and 72 h fermentation, *n* = 6 for each substrate) are shown by *p*-values (non-parametric Student's *t*-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Beta-diversity of microbiota presented by the Principal Coordinate Analysis (PCoA) plots. (A) Diversity across the substrates FiberBind 400 (FB, red), resistant starch (RS, blue) and native starch (NS, green) using weighted and unweighted UniFrac distance matrices. (B) Diversity over time using weighted UniFrac distance matrix. PCoA plots show sample grouping according to fermentation time (0 h blue, 24 h red, 48 h green, 56 h yellow and 72 h pink): 0–72 h (middle plot); 0 and 24 h (plot to the left); 48, 56 and 72 h (plot to the right). Microbiota samples were collected from two independent TIM-2 fermentations of each substrate and analyzed using Illumina NextSeq 500 Sequencing System. PCoA plots were constructed for the samples after 48, 56 and 72 h fermentation ($n = 6$ for each substrate). The ellipse center indicates group means. Group differences were tested by ANOSIM as shown by R-values and p-values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Erysipelotrichaceae (*Eubacterium bioforme*), were not significantly different between FiberBind 400 and starches.

3.6. Production of SCFA and BCFA

Acetate was the most abundant fatty acid produced in fermentations of FiberBind 400 (61 mmol), followed by propionate (27 mmol) and n-butyrate (21 mmol) (Table 1). The cumulative amounts of total SCFA, total BCFA and isovalerate in fermentations of FiberBind 400 were lower compared to resistant starch ($p < .05$), while the differences between the substrates in production of other individual fatty acids were statistically insignificant.

4. Discussion

4.1. FiberBind 400 improved survival of *Lactobacillus* spp.

We explored the prebiotic potential of potato fiber FiberBind 400, including the ability to improve survival of probiotic *Lactobacillus* strains exposed to simulated gastric stresses and to modulate the gut microbiota using the TIM-2 colon model. Reference substrates for the experiments were resistant cross-linked potato starch, commonly classified as a prebiotic fiber (X. Yang et al., 2017) and rapidly digestible starch from potato. We observed that bacterial viability in gastric solution was strain-dependent and generally improved by potato fiber, highest in case of *L. fermentum* PCC® and *L. rhamnosus* LGG®. Protective effect of potato fiber might be especially advantageous for *L. rhamnosus*

LGG®, otherwise highly sensitive to simulated gastric conditions. The positive effect of FiberBind 400 on viability of *L. reuteri* RC-14® and *L. paracasei* F-19®, though only observed in the first part of incubation (1–2 h), might be essential for bacterial survival, regarding the relatively short transit time in the stomach (Read, Cammack, Edwards, & Holgate, 1982). Concurrently, the impact of resistant starch and native starch on bacterial viability was insignificant, indicating that non-starch components of potato fiber, e.g., pectin, might be involved in protection mechanisms. This assumption is based on prior evidence, showing that resistance of *Lactobacillus* spp. to gastric juice might be improved by treatment with pectins from citrus fruits and sugar beet (Larsen et al., 2018). Moreover, enhancement of bacterial viability by FiberBind 400 was similar or even higher compared to pectins. Bacterial cell surface factors and the nature of molecular interactions, underlying the beneficial effects of potato fiber, need to be clarified.

4.2. Shifts in microbiota mediated by FiberBind 400 and their association with gut health

In addition to non-resistant starch, easily degradable by most of the gut commensals, FiberBind 400 is rich in fibers (cell wall polysaccharides and resistant starch) utilized by specific bacterial species. Complex composition of potato fiber resulted in stimulation of various bacterial populations and led to higher diversity of microbiota compared to starches. The major assimilators of fibers belonging to *Bacteroides* and *Prevotella* within the phylum *Bacteroidetes* were generally increased in TIM-2 fermentations. The evidence on health effects

Fig. 4. Heatmap showing relative abundances of bacterial taxa in TIM-2 fermentations of FiberBind 400 (FB), resistant starch (RS) and native starch (NS). Microbiota samples were collected from two independent fermentations of each substrate and analyzed by Illumina NextSeq 500 Sequencing System. Bacterial abundances at fermentation start (0 h) are shown by mean values ($n = 6$). Differences in abundances were evaluated for combined data (48, 56 and 72 h; $n = 6$ for each substrate) using the Wilcoxon Rank Sum test and Bonferroni multiplicity correction ($p < .05$). Abundances of bacterial taxa significantly changed by FiberBind 400 compared to 0 h are presented by numbers. Significant differences between FiberBind 400 and resistant starch or native starch are denoted by letters RS or NS, respectively. Taxa denoted as “Other” indicate ambiguity in the assignment; taxa in square brackets indicate a proposed taxonomy. The colour key presents the log-transformed mean values of abundances.

of *Prevotella*, stimulated highest by FiberBind 400 is rather inconsistent. Thus, Kovatcheva-Datchary et al. (2015) found that *Prevotella* contributed to improved glucose metabolism in humans after intake of a fiber-rich diet (Kovatcheva-Datchary et al., 2015), while Pedersen et al. (2016) reported induction of insulin resistance and glucose intolerance in humans and mice by *P. copri*, the most abundant species of *Prevotella* (Pedersen et al., 2016). A unique feature of *Bacteroidetes* genomes is the presence of polysaccharide utilization loci (PULs), encoding the

carbohydrate active enzymes (CAZymes) which facilitate the breakdown of complex polysaccharides (Grondin, Tamura, Déjean, Abbott, & Brumer, 2017). Interestingly, potato fiber was most efficient to support growth of *B. ovatus*, whereas resistant starch was a preferable substrate for *B. uniformis*. Species-specific utilization of dietary fibers was, most probably, related to the differences in CAZymes activities between the species. Supporting this assumption, preferential utilization of substrates have been reported for *Bacteroides* species grown in mixed

Table 1

Cumulative production of short chain fatty acids (SCFA) and branched chain fatty acids (BCFA) after 72 h fermentation of FiberBind 400, resistant starch and native starch in TIM-2 colon model.

Fatty acids	FiberBind 400, mmol ^a	Resistant starch, mmol	Native starch, mmol
Acetate	61.0 ± 3.6	59.6 ± 5.6	59.1 ± 10.8
Propionate	26.9 ± 1.5	31.4 ± 4.1	23.5 ± 3.3
Butyrate	20.9 ± 0.6	27.5 ± 4.1	22.4 ± 0.5
Iso-butyrate	5.3 ± 0.1	5.1 ± 0.8	5.6 ± 0.4
Iso-valerate	14.5 ± 0.4*	19.7 ± 0.3	16.0 ± 5.1
Total SCFA	108.7 ± 4.4*	118.4 ± 3.2	104.9 ± 14.6
Total BCFA	19.8 ± 0.3*	24.9 ± 0.5	21.5 ± 5.5

^a Mean values (± SD) were determined in two independent TIM-2 experiments (lumen and dialysate samples). The amount of fatty acids at the start of fermentations was set to zero. Asterisks shows significant differences between FiberBind 400 and resistant starch (paired Student t-test, *p < .05). Differences between FiberBind 400 and native starch were insignificant.

cultures (Hamaker & Tuncil, 2014; Tuncil et al., 2017). Along with the members of the phylum *Bacteroidetes*, the increased abundances of *Clostridiaceae*, *Ruminococcaceae* and *Clostridium* within the phylum *Firmicutes*, and the family *Enterobacteriaceae* within phylum *Proteobacteria* in TIM-2 experiments can be related to their ability to break down and utilize complex polysaccharides (Abbott & Boraston, 2008; Thomassen, Vignæs, Licht, Mikkelsen, & Meyer, 2011; Venkataraman et al., 2016). Utilization of the products of potato fiber degradation, or the metabolic cross-feeding interactions (Flint, Scott, Duncan, Louis, & Forano, 2012), is most probably the main factor favoring growth of *Lachnospira*, *Butyrivibrio*, *Oscillospira*, and *Christensenellaceae* in potato fiber fermentations. These bacterial populations are commonly associated with a healthier gut environment and found to be predominant in fecal samples from lean individuals over obese (Aguirre & Venema, 2015; Goodrich et al., 2014; Menni et al., 2017). Concurrently, reduction in butyrate producing bacteria *F. prausnitzii*, *Roseburia*, *Eubacterium* spp., and *Blautia*, mediated by potato fiber, might be considered unfavorable, regarding their protective role in gut inflammation and metabolic diseases (Cani, 2013; Qin et al., 2012; Upadhyaya, McCormack, Fardin-kia, & Juenemann, 2016; Verdam et al., 2013). Another characteristic feature of FiberBind 400 fermentations is an extensive increase in the members of *Proteobacteria*, specifically *Desulfovibrio* species. The role of *Desulfovibrio* in the gut health is still unclear. It was suggested that *Desulfovibrio* have a positive impact in cancer prevention (Rudi, Ludvigsen, Dirven, & Steffensen, 2017), whereas other studies highlighted the adverse effects of *Desulfovibrio* associated with ulcerative colitis (Lennon et al., 2014; Ma, Yu, Zhao, Zhang, & Zhang, 2018). The genus *Bifidobacterium* is known to include fiber degrading species, particularly effective in starch utilization (Lockyer & Nugent, 2017; Thomassen et al., 2011); accordingly, it was highly stimulated by native starch and moderately increased by other substrates in this study. An increase in *Bifidobacterium* is commonly referred to as beneficial, having the potential to counteract obesity, Crohn's disease and ulcerative colitis (Callaghan & Van Sinderen, 2016).

Consistent with our results, higher abundances of fecal *Oscillospira*, *Bacteroides*, *Prevotella*, *Bifidobacterium*, *Parabacteroides distasonis*, *Desulfovibrio* and *Mogibacterium*, as well as lower abundance of *P. copri* have been reported in human trials after consumption of resistant starches (Alfa et al., 2018; Upadhyaya et al., 2016). *Bifidobacterium* was also stimulated by potato fiber diets in rats (Paturi et al., 2012; Young et al., 2012). Some other studies, however, reported different results as, e. g., increased numbers of fecal *Faecalibacterium* and *Blautia* by potato fiber given to dogs (Panasevich et al., 2015). Discrepancies between the studies can be due to the differences in initial microbiota and composition of potato fibers which can affect efficiency of substrate utilization (Pastuszewska et al., 2009). Based on extensive evidence of structure-function relationship in microbiota fermentations, we expect that

fermentation of FiberBind 400 would mediate unique cross-feeding microbial interactions and specific shifts in microbiota. In the previous TIM-2 experiments with structurally diverse pectins, we found that microbiota composition was associated with degree of methylation of pectins (Larsen et al., 2019). Increased abundances of *Lachnospira*, *Ruminococcus*, *Prevotella*, *B. ovatus*, and *Desulfovibrio* spp., as well as reduction of *F. prausnitzii* and *P. copri* in fermentations of FiberBind 400, were observed specifically in fermentations of low-methoxylated (LM) pectins. Supporting these results, pectins from potato fibers have been categorized as LM pectins with methylation degree of 30% (Yang, Mu, & Ma, 2018). Similarities between potato fiber and LM pectins indicate that the pectin component seems to be important for microbiota shaping in fermentations of FiberBind 400.

Metabolism of potato fiber and starches by the microbiota was evaluated by measurements of short-chain and branched-chain fatty acids. SCFA have various beneficial functions, playing a key role in the gut immunity, glucose homeostasis, cell proliferation, metabolic processes, etc. (Wang et al., 2019). BCFA, on the other hand, are associated with insulin resistance and cardio-metabolic diseases and commonly regarded as undesirable (Brial, Le Lay, Dumas, & Gauguier, 2018). Production of total fatty acids by FiberBind 400 was lower compared to resistant starch, indicating slower fermentability of potato fiber. Differences between the substrates in production of individual short-chain and branched-chain fatty acids were mostly insignificant in this study. This finding was unexpected, considering substrate-dependent changes in butyrate and propionate producing bacteria within *Lachnospiraceae*, *Blautia*, *Bacteroides*, *Prevotella* and *Ruminococcus* (Louis & Flint, 2017). Accordingly, similar levels of SCFA and BCFA produced in TIM-2 experiments by different dietary fibers have been reported previously, and it might be attributed to functional redundancy of the microbiota and the metabolic cross-feeding interactions (Aguirre, De Souza, & Venema, 2016; Larsen et al., 2019).

5. Conclusions

This study suggests that potato fiber has a potential to improve survival of probiotic strains of *Lactobacillus* spp. at gastric conditions, depending on the strain. The protective ability of FiberBind 400 might support efficient delivery of viable probiotics through the stomach when used as, e.g., a component of probiotic containing food. Experiments in the TIM-2 colon model revealed the bifidogenic effect and other shifts in microbiota, suggesting that diet supplementation with FiberBind 400 can selectively stimulate commensals in the gut, among them, health-promoting bacteria. Like many other dietary fibers, potato fiber fermentation led to the production of SCFAs, favorable for colonic health. The overall results indicate that FiberBind 400 has a prebiotic potential and facilitate application of potato fiber as a prebiotic food ingredient with the hypothesized biological and health benefits. Knowledge generated in this study contribute to the growing recognition of the role of fiber products as dietary prebiotics, specifically, in maintaining more balanced microbial communities in the gut. Further extensive human studies, including the impact on microbial dysbiosis associated with a diseased gut, are required to clarify the health benefits of FiberBind 400.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2019.108644>.

Ethical approval

Studies using fecal donations from healthy volunteers do not require medical ethical committee approval in The Netherlands, since they are considered as non-invasive. Nevertheless, all participants provided informed consent. Results in this manuscript are referred to a pool fecal inoculum and do not directly refer to a particular person.

Declaration of competing interests

The authors declare that they have no competing interests.

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