Anaerobe 42 (2016) 60–66

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

# In vitro analysis of partially hydrolyzed guar gum fermentation on identified gut microbiota

Justin Carlson [a], Trevor Gould [b], Joanne Slavin [a, *]

[a] Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave St. Paul, MN 55108, United States
[b] Informatics Institute, University of Minnesota, 101 Pleasant St, Minneapolis, MN 55455, United States

ARTICLE INFO

*Article history:*
Received 14 June 2016
Received in revised form
22 August 2016
Accepted 22 August 2016
Available online 30 August 2016

Handling Editor: Elisabeth Nagy

*Keywords:*
PHGG
Microbiome
Prebiotic
Fermentation
Microbiota

ABSTRACT

*Background:* Prebiotic dietary fibers resist digestion in the upper gastrointestinal tract and allow for stimulation of bacteria in the distal intestine and colon. Stimulation of bacteria among different individuals varies greatly, depending on a wide range of variables.
*Objective:* To determine the range of differences in response between individuals, a preclinical in vitro fermentation was conducted with six fecal donors. The primary objective was to compare the fecal microbiota of six individuals at baseline, 12 h and 24 h post-exposure to partially hydrolyzed guar gum (PHGG).
*Method:* Fecal donations were collected from six healthy individuals consuming a non-specific Western diet, free of antibiotic treatments in the past year, not affected by any GI diseases and not consuming any probiotic or prebiotic supplements. Fecal samples were exposed to 0.5 g of PHGG and measured for bacterial changes at 0, 12 and 24 h based on 16S rRNA sequencing.
*Results: Parabacteroides* increased from 3.48% of sequence reads to 10.62% of sequence reads after 24 h ($p = 0.0181$) and Bacteroidetes increased from 45.89% of sequence reads to 50.29% of sequence reads ($p = 0.0008$).
*Conclusions:* PHGG stimulates growth of *Parabacteroides*, a genus of bacteria that have been inversely associated with IBS and ulcerative colitis. PHGG provides stimulation of beneficial Bacteroidetes (*Bacteroides* and *Parabacteroides*), which may be correlated with many positive health markers and outcomes. PHGG is a prebiotic dietary fiber that is readily fermentable.



## 1. Introduction

Fiber consumption in the U.S. is approximately half of the recommended intake, with the average U.S. individual only consuming about 17 g/d [1]. Dietary fiber with prebiotic capacity offers a healthful, practical way to bridge the gap between consumption and recommended intake. Dietary fiber supplements can offer some of the same physiological benefits as dietary fiber found intact in foods [2]. When consumed in adequate levels, dietary fiber has been shown to help maintain a healthy body weight [3], improve cardiovascular health [4–6], support overall digestive health [7], and support the overall growth of the intestinal microbiota [8].

The intestinal microbiota have a tremendous impact on overall health, and have been recently shown to have a significant impact on the host's metabolism, immune system capacity and many other pathways affecting overall host health [9–11]. Recent studies have also found many associations between many diseases and the host's microbial composition, including: metabolic syndrome, diabetes, and many gastrointestinal diseases [12–15]. The gut microbiome and it's correlation to health and disease is a quickly revolving, dynamic area of research. Next-generation sequencing and other advanced sequencing technologies have provided new insight into this rapidly expanding field, and have allowed efficient and effective ways to analyze the thousands of diverse taxa within the human gastrointestinal tract [16,17].

Partially hydrolyze guar gum (PHGG) is a dietary fiber made from the controlled hydrolysis of guar gum, composed of both mannose and galactose monomers, and is commonly consumed as both a dietary fiber supplement, as well as in foods. PHGG has been shown to alleviate irritable bowel syndrome (IBS) due to its non-gelling effects [18] and has been shown in randomized, cross-

* Corresponding author.
*E-mail address:* jslavin@umn.edu (J. Slavin).

http://dx.doi.org/10.1016/j.anaerobe.2016.08.006
1075-9964/

over clinical studies to increase satiety [19,20]. In a four-week clinical feeding study where participants consumed 20 g/d of PHGG researchers found an overall decrease in total serum cholesterol, an increase in fecal weight and a lower fecal pH without influencing key nutrient absorption. In vitro models have shown that PHGG supports the growth of *Bifidobacterium* and *Lactobacillus*, two genera of bacteria that have been associated with many health outcomes [21,22].

This paper is a follow-up of previously published work from our laboratory that evaluated the differences in fermentation rates between six individuals' fecal microbiota all exposed to PHGG in an in vitro fermentation model, with the primary objective of showing the differences in short chain fatty acid (SCFA) synthesis and total gas production, and found over a 2-fold difference in total SCFA production in 24 h [23]. The current study has the objective of determining key changes and individual differences among six individual's fecal microbiota exposed to PHGG in an in vitro fermentation model. To the authors' knowledge, this is the first in vitro model with six individuals to show the short-term effects of PHGG on the fecal microbiota.

## 2. Materials

Fiber analyzed in this study was partially hydrolyzed guar gum (Benefibra™, Novartis Consumer Health Spa Origgio, Varese, Lombardy, Italy). PHGG is a dietary fiber made from the controlled hydrolysis of guar gum from the guar plant *Cyamoposis tetragonolobus*, composed of both mannose and galactose monomers. PHGG is a soluble, non-viscous fiber often incorporated into both foods and beverages. Chemical reagents used were provided by ThermoFisher Scientific (ThermoFisher Scientific Inc., Waltham, MN, USA), Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and Oxyrase (Oxyrase Inc., Mansfield, OH, USA).

## 3. Methods

### 3.1. Donor information

Healthy fecal donors (male and female) were selected to represent a wide range in age (21–68) and BMI (19.5–33.6) for representative samples from a population (Table 1). Donors were selected based on screening questionnaire and previous participation.

### 3.2. Fecal collection

Fecal samples were collected from six healthy volunteers (5 males, 1 female) under anaerobic conditions from individuals (ages 21–68) consuming non-specific Western diets, free of any antibiotic treatments in the last year, not affected by any GI diseases and not consuming any probiotic or prebiotic supplements. Fecal samples were anaerobically collected within 10 min of the start of the fermentation, and homogenized immediately upon collection. All data and samples collected were done in accordance with University of Minnesota policies and procedures.

**Table 1**
Demographic characteristics of six fecal donors.

| | Donor 1 | Donor 2 | Donor 3 | Donor 4 | Donor 5 | Donor 6 |
|---|---|---|---|---|---|---|
| Age | 31 | 68 | 60 | 24 | 22 | 21 |
| Sex | Male | Male | Female | Male | Male | Male |
| BMI | 23.7 | 33.6 | 19.5 | 26.3 | 24.7 | 23.0 |

### 3.3. Fermentation

Fiber samples (0.5 g) were hydrated in 40 mL of prepared sterile tricase peptone fermentation media in 100 mL serum bottles, capped, and incubated for 12 h at 4 °C. Following incubation, serum bottles were transferred to a circulating water bath at 37 °C and allowed to incubate for 2 h. Post-collection, fecal samples were mixed using a 6:1 ratio of phosphate buffer solution to fecal sample. After mixing, obtained fecal slurry was combined with prepared reducing solution (2.52 g cysteine hydrochloride, 16 mL 1 N NaOH, 2.56 g sodium sulfide non anhydride, 380 mL DD $H_2O$) at a 2:15 ratio. 10 mL of the prepared fecal inoculum was added to each of the serum bottles, 0.8 mL Oxyrase® was added, flushed with $CO_2$, sealed, and then immediately placed in a 37 °C circulating water bath. Samples were prepared in triplicate and analyzed at 0, 12 and 24 h. Upon removal at each time point, total gas volume was measured. Then samples were divided into aliquots for analysis and 1 mL of copper sulfate (200 g/L) was added to cease fermentation. All samples were immediately frozen and stored at −80 °C for further analysis.

### 3.4. DNA extractions

Fecal bacteria DNA from the in vitro system were extracted using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc.) following provided operating instruction, including bead beating for 20 min.

### 3.5. Primary/secondary PCR amplification

The V1–V3 region of the 16S rRNA was amplified using a two-step PCR protocol. The primary amplification was done using an ABI7900 qPCR machine. The following recipe was used: 3 μl template DNA, 0.48 μl nuclease-free water, 1.2 μl 5× KAPA HiFi buffer (Kapa Biosystems, Woburn, MA), 0.18 μl 10 mM dNTPs (Kapa Biosystems, Woburn, MA), 0.3 μl DMSO (Fisher Scientific, Waltham, MA), 0.12 μl ROX (25 μM) (Life Technologies, Carlsbad, CA), 0.003 μl 1000× SYBR Green, 0.12 μl KAPA HiFi Polymerase (Kapa Biosystems, Woburn, MA), 0.3 μl forward primer (10 μM), 0.3 μl reverse primer (10 μM). Cycling conditions were: 95°C for 5 min, followed by 20 cycles of 98°C for 20 s, 55°C for 15 s, and 72°C for 1 min. The primers for the primary amplification contained both 16S-specific primers (V1_27F and V3_V34R), as well as adapter tails for adding indices and Illumina flow cell adapters in a secondary amplification. The following primers were used (16S-specific sequences in bold): Meta_V1_27F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGAGTTTGATCMTGGCTCAG)** and Meta_V3_534R (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATTACCGCGGCTGCTGG)**.

The amplicons from the primary PCR were diluted 1:100 in sterile, nuclease-free water, and a second PCR reaction was set up to add the Illumina flow cell adapters and indices. The secondary amplification was done on a fixed block BioRad Tetrad PCR machine using the following recipe: 5 μl template DNA, 1 μl nuclease-free water, 2 μl 5× KAPA HiFi buffer (Kapa Biosystems, Woburn, MA), 0.3 μl 10 mM dNTPs (Kapa Biosystems, Woburn, MA), 0.5 μl DMSO (Fisher Scientific, Waltham, MA) 0.2 μl KAPA HiFi Polymerase (Kapa Biosystems, Woburn, MA), 0.5 μl forward primer (10 μM), 0.5 μl reverse primer (10 μM). Cycling conditions were: 95°C for 5 min, followed by 10 cycles of 98°C for 20 s, 55°C for 15 s, 72°C for 1 min, followed by a final extension at 72°C for 10 min. The following indexing primers were used (X indicates the positions of the 8 bp indices): Forward indexing primer: AATGATACGGCGACCACCGAGATCTACACXXXXXXXXTCGTCGGCAGCGTC and Reverse indexing primer: CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTCTCGTGGGCTCGG.

### 3.6. Normalization and sequencing

The samples were normalized using a SequalPrep capture-resin bead plate (Life Technologies, Carlsbad, CA) and pooled using equal volume. The final pools were quantified via PicoGreen dsDNA assay (Life Technologies, Carlsbad, CA) and diluted to 2 nM 10 μl of the 2 nM pool was denatured with 10 μl of 0.2 N NaOH, diluted to 8 pM in Illumina's HT1 buffer, spiked with 15% phiX, heat denatured at 96°C for 2 min, and sequenced using a MiSeq 600 cycle v3 kit (Illumina, San Diego, CA).

### 3.7. Sequence processing and analysis

Generated sequence data was processed and analyzed using QIIME [24]. Fastq sequence data was processed with the University of Minnesota's gopher-pipeline for metagenomics [25]. Sequence data had adapters removed and sliding quality trimming window by Trimmomatic [26]; primers removed and overlapping reads merged by Pandaseq [27]. Within QIIME, chimera checking done by chimera slayer, Open reference OTU picking completed with Usearch61, taxonomic identification using GreenGenes (Version 13.8) reference database, rarefied to 14,393 sequences per sample. Analysis was performed using R (R Development Core Team, 2012).

### 3.8. Statistical analysis

All statistical analysis was performed using R software (R Development Core Team, 2012). Differences in means were determined using the Kruskal-Wallis ANOVA test, testing the null hypothesis that the location parameter of the groups of abundances for a given OTU is the same. Multiple comparisons were corrected using the FDR procedure. Significance was set for $p$-values <0.05.

### 3.9. Consent

Voluntary informed consent was obtained from all fecal donors prior to this study according to University of Minnesota policies and procedures.

## 4. Results

Sequencing of the 16S rRNA gene was conducting using Illumina MiSeq 2000 sequencing platforms at the University of Minnesota Genomics Center. From the 6 fecal samples analyzed from the in vitro system across 24 h of fermentation (6 fecal samples × 3 time points × 3 technical replicates = 54 samples analyzed), there were >30 million total sequences generated, representing 11 bacteria phyla and 416 OTUs at a 97% similarity threshold. Changes in fecal microbiota were analyzed for all six fecal donors in the in vitro fermentation system at baseline, 12 and 24 h. Taxa with greater than 0.05% overall abundance were summarized (Table 2). At baseline, Bacteroidetes and Firmicutes were the most abundant phyla of bacteria, consisting of approximately 90% of all sample reads. Proteobacteria was the third most abundant phyla identified, consisting of approximately 4% of the sample reads. Actinobacteria and Verrucomicrobia accounted for less than 1% of all sequence reads.

Across the 24 h of analysis, overall abundance of sequences of Bacteroidetes increased significantly ($p < 0.001$) from approximately 45.9% of sequence reads at baseline to approximately 50.3% of sequences at 24 h. Firmicutes accounted for approximately 44.9% of total sequences at baseline, which decreased to 40.4% of sequence reads at 24 h ($p = 0.0527$), trending towards significance. Verrucomicrobia accounted for 0.51% of sequence reads at baseline, and increased significantly over 24 h to 0.82% of sequence reads at 24 h ($p < 0.001$), although still accounted for less than 1% of all sequence reads. Similar shifts in abundant phyla identified were similar for all fecal donors (Fig. 1).

**Table 2**
Combined changes across 24 h of fermentation for identified abundant taxa. Significance was determined using the Kruskal-Wallis ANOVA test, testing the null hypothesis that the location parameter of the groups of abundances for a given OTU is the same, with multiple comparisons corrected using the FDR procedure. Samples were analyzed between differentially represented OTUs for significant overall changes, not between any two time points.

| Targeted taxa (phlyum and genus) | Baseline | 12 h | 24 h | $p$-value |
|---|---|---|---|---|
| Actinobacteria | 0.97 | 0.79 | 1.77 | 0.1294 |
| *Collinsella* | 0.78 | 0.68 | 1.64 | 0.0986 |
| *Adlercreutzia* | 0.03 | 0.02 | 0.02 | 0.6104 |
| Bacteroidetes | 45.89 | 52.32 | 50.29 | 0.0008 |
| *Bacteroides* | 27.12 | 36.13 | 33.05 | 0.1455 |
| *Parabacteroides* | 3.48 | 10.18 | 10.62 | 0.0181 |
| *Prevotella* | 0.83 | 0.30 | 0.18 | 0.6385 |
| *Odoribacter* | 0.53 | 0.14 | 0.18 | 0.0002 |
| *Paraprevotella* | 0.21 | 0.04 | 0.01 | 0.9877 |
| *YRC22* | 0.18 | 0.03 | 0.02 | 0.6477 |
| *Butyricimonas* | 0.13 | 0.03 | 0.04 | 0.0323 |
| Firmicutes | 44.87 | 39.33 | 40.43 | 0.0527 |
| *Blautia* | 2.48 | 1.34 | 1.32 | 0.1763 |
| *Faecalibacterium* | 1.59 | 1.10 | 0.93 | 0.0043 |
| *Ruminococcus* | 1.52 | 1.78 | 2.12 | 0.9829 |
| *Coprococcus* | 0.77 | 0.78 | 0.55 | 0.2631 |
| *Oscillospira* | 0.74 | 0.57 | 0.63 | 0.2303 |
| *Lachnospira* | 0.55 | 0.08 | 0.09 | <0.0001 |
| *Phascolarctobacterium* | 0.51 | 2.32 | 2.31 | 0.0011 |
| *Dorea* | 0.27 | 0.13 | 0.27 | 0.0535 |
| *Streptococcus* | 0.19 | 0.06 | 0.07 | 0.0590 |
| *Dialister* | 0.18 | 0.14 | 0.16 | 0.5368 |
| *Clostridium* | 0.16 | 0.06 | 0.06 | 0.1235 |
| *Eubacterium* | 0.13 | 0.45 | 0.68 | 0.2619 |
| *Veillonella* | 0.12 | 0.07 | 0.08 | 0.6247 |
| *Ruminococcus* | 0.11 | 0.08 | 0.11 | 0.4163 |
| *Catenibacterium* | 0.08 | 0.17 | 0.16 | 0.5981 |
| *Roseburia* | 0.08 | 0.12 | 0.06 | 0.0906 |
| *Turicibacter* | 0.08 | 0.01 | 0.02 | 0.0239 |
| *SMB53* | 0.07 | 0.02 | 0.03 | 0.1632 |
| *Anaerostipes* | 0.06 | 0.75 | 0.94 | 0.9189 |
| Proteobacteria | 4.09 | 5.64 | 5.62 | 0.5850 |
| *Sutterella* | 2.36 | 3.97 | 3.60 | 0.6508 |
| *Bilophila* | 0.14 | 0.05 | 0.08 | <0.0001 |
| *Haemophilus* | 0.10 | 0.05 | 0.03 | 0.0744 |
| *Oxalobacter* | 0.06 | 0.009 | 0.01 | 0.2984 |
| Verrucomicrobia | 0.51 | 0.90 | 0.81 | <0.0001 |
| *Akkermansia* | 2.52 | 0.58 | 0.56 | <0.0001 |

At the genus level, *Bacteroides* was the most abundant genus in all samples, consisting of 27.1% of sequence reads at baseline, which increased to 33.05% of all reads at 24 h ($p = 0.14$) (Table 1). *Parabacteroides* had the greatest stimulation, increasing from 3.48% of sequence reads at baseline to 10.18% of sequence reads at 12 h and 10.62% at 24 h ($p = 0.0181$). *Odoribacter* decreased significantly from baseline compared to 24 h of fermentation, decreasing from 0.54% at baseline to 0.18% after 24 h of fermentation ($p = 0.002$). *Phascolarctobacterium* increased significantly from 0.51% at baseline to 2.31% after 24 h ($p = 0.0011$). The 19 most abundant genera of bacteria composed >99% of all sequences analyzed (Fig. 2), which we identified in all 6 fecal donors.

Comparing ratios of Bacteroidetes:Firmucutes, each individual's fecal microbiota responded much differently to PHGG after 12 h and 24 h post-exposure (Fig. 3). The average percent reads of Firmicutes at baseline was 44.87% of total reads, although this varied greatly from donor to donor, from as low as 29% of total reads, to as high as 47%. After 12 h of fermentation, some donors' microbiota

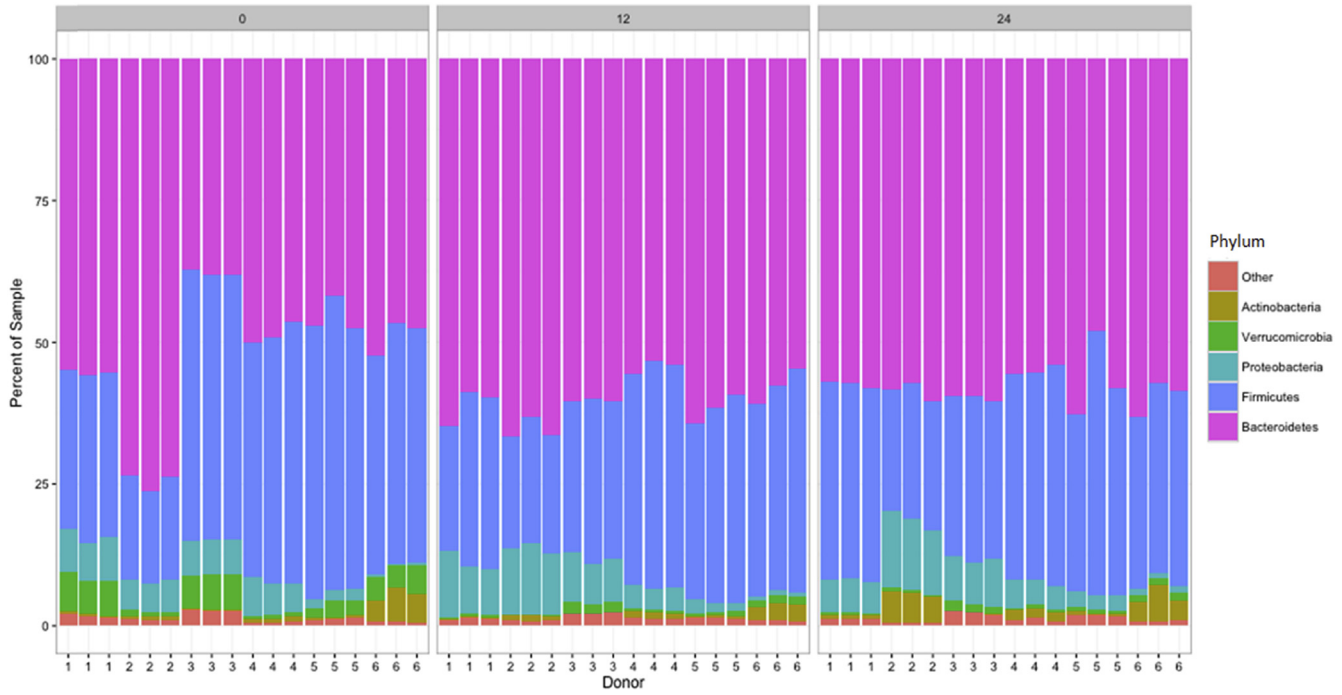


**Fig. 1.** Identified abundant phyla based on percent of sequence reads at 0, 12 and 24 h of fermentation of PHGG for six fecal donors. Specified genera consisted of >99% of all sample reads for each individual donor. Technical replicates listed for each fecal donor at each specified time of analysis. *PHGG (partially hydrolyzed guar gum).

populations of Firmicutes increased, while some decreased as much as 10% and some were unaffected. Firmicutes and Bacteroidetes population fluctuations were primarily specific to each donor, with some overall similar trends.

Differences in analyzed microbiota were analyzed between donors and across reference fermentation time points. The Unweighted Unifrac beta diversity PCoA plot, measuring dissimilarity between all sequence reads based their generated 99% OTU composition, showed that across all measured time points that samples were most similar to each donor, and not their time of analysis (Fig. 4). Donors 1–3 (ages 31–68) were also more similar to one another than donors 4–6 (ages 21–24).

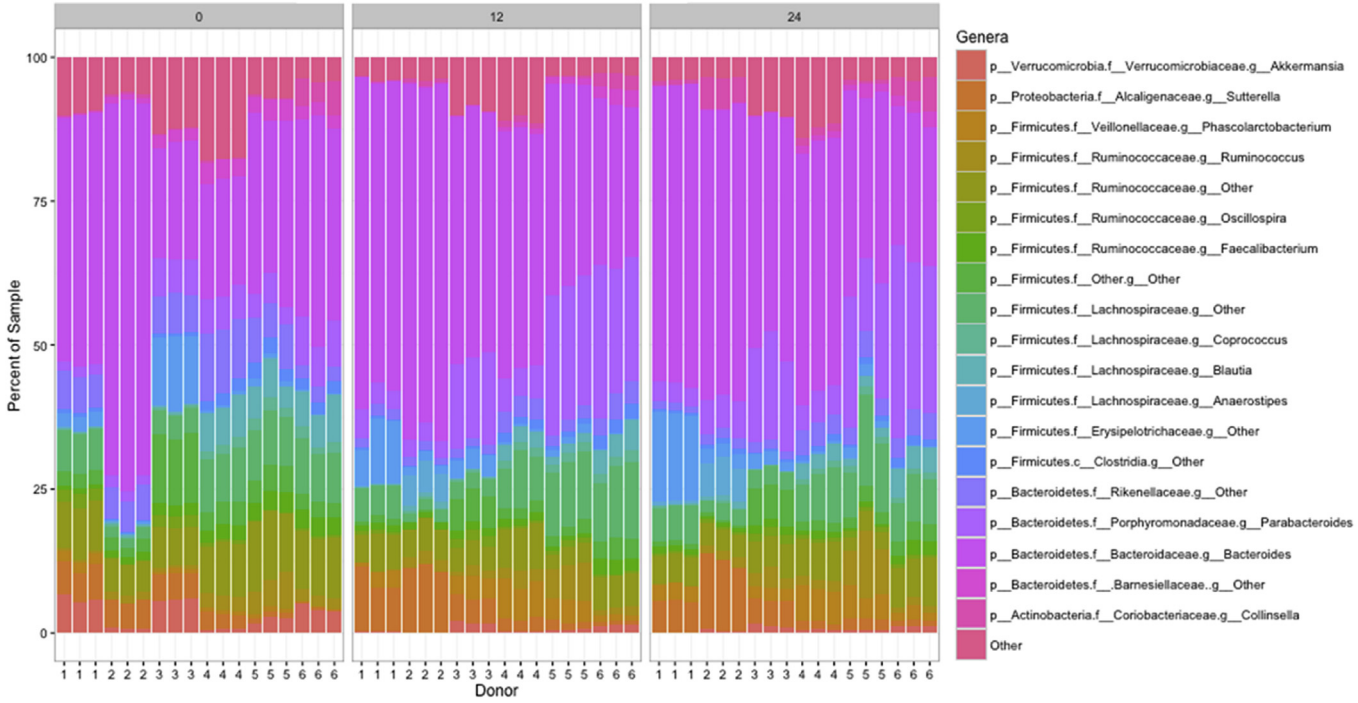


**Fig. 2.** Identified abundant genera at 0, 12 and 24 h of fermentation of PHGG for six fecal donors. Specified genera consisted of >99% of all sample reads for each individual donor. Technical replicates listed for each fecal donor. *PHGG (partially hydrolyzed guar gum).

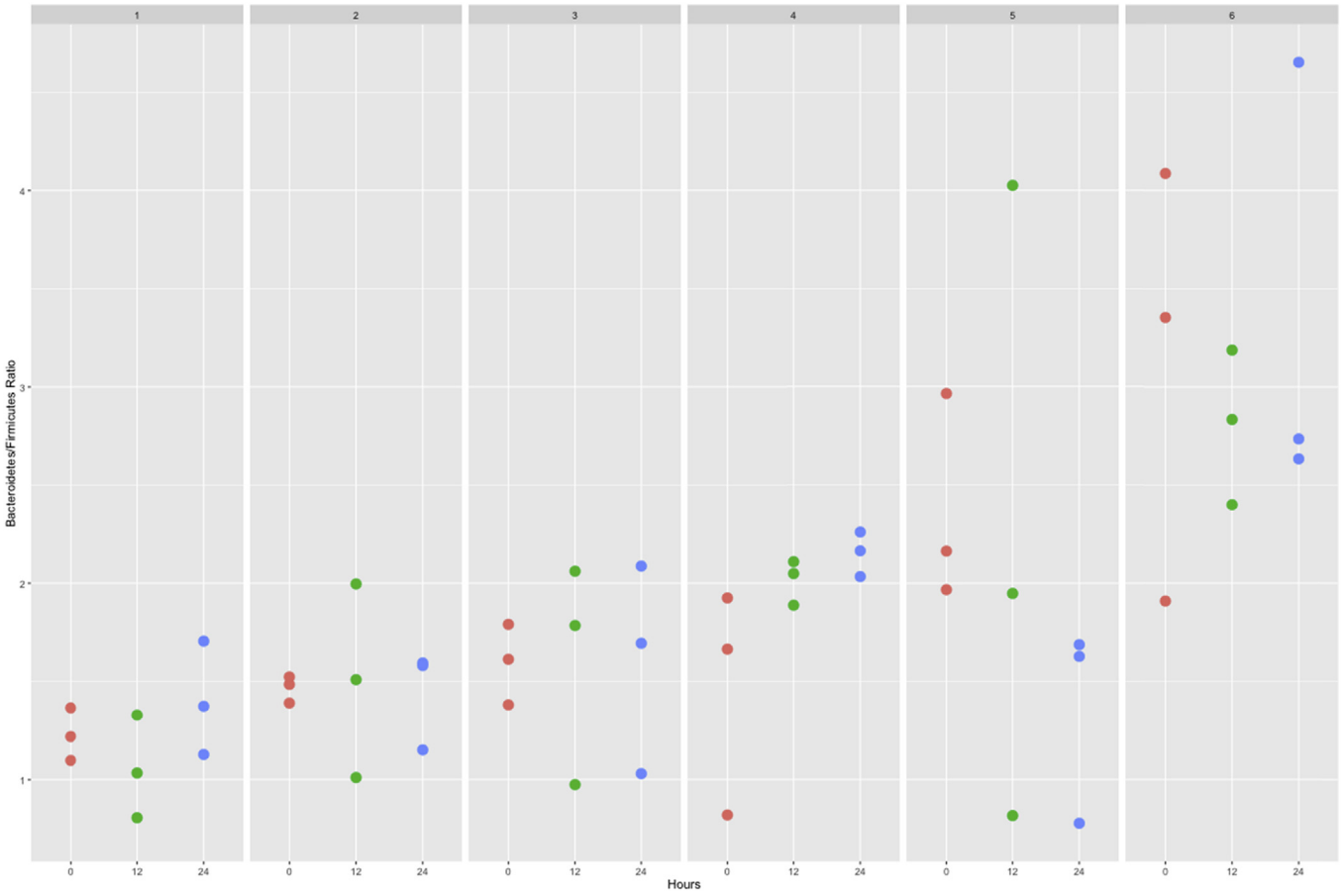


**Fig. 3.** Fluctuations in ratios of Bacteroidetes:Firmicutes at 0, 12 and 24 h of analysis based on percentage of total sequence reads for six fecal donors analyzed. Red (0 h), green (12 h) and blue (24 h) indicate length of sample fermentation in vitro.

## 5. Discussion

The increase of *Parabacteroides* was the most significant increase among all identified taxa, increasing from 3.48% of sequence reads at baseline to 10.62% of sequence reads after 24 h of fermentation. In a clinical study, oral administration of *P. distasonis* has been shown to reduce severity of intestinal inflammation in induced acute and chronic colitis in murine models due to the modulation of both immunity and microbiota factors [28]. *Parabacteroides* has been identified as one of the most abundant genera of bacteria in the human gastrointestinal tract [29]. *Parabacteroides* has also been shown to be present in higher concentrations in healthy controls compared to patients with IBS or ulcerative colitis, and it has been speculated that this genera of bacteria may play an influential role in the pathogenesis of both diseases [30]. *Parabacteroides* stimulation has been considered to have a prebiotic effect due to their preference to perform saccharolytic activity instead of proteolytic activity [8,31].

PHGG has been extensively reviewed for the treatment of IBS [32,33]. Briefly, PHGG has been shown to offer prebiotic activity due to its increase in colonic SCFA production and stimulation of *Lactobacillus* and *Bifidobacterium* [34,35] in two studies utilizing culture-dependent and florescent in situ hybridization (FISH) methods. Baseline *Bifidobacterium* and *Lactobacillus* sequence reads were less than 0.01% of all sequence reads for each respective genus in all six donors. Low bifidobacteria and lactobacilli counts could be due to a wide variety of influential factors (age, stress, environmental components) [36–40] or potentially due to the age of fecal donors [39]. Other studies have found bifidobacteria to be found in very low quantities, if found at all [41,42]. Metagenomic 16S rRNA studies have often found under representation of bifidobacteria due to potential biases in PCR primers and amplification, and have found potential solutions using *cpn60*-based methods [43]. Because of the low concentrations of these genera of bacteria in most populations, it is imperative to analyze other abundant taxa of bacteria using next-generation sequencing methods. PHGG fermentation often doesn't support growth of these genera of bacteria in in vitro models because it is the degraded products of PHGG that support growth of these bacteria, which aren't formed in in vitro models [35]. PHGG is enzymatically broken down into low molecular weight galactomannan, which is the primary carbon source for intestinal bacteria [44–46]. Galactomannan formation from PHGG consists of a mannose backbone with galactose side groups (mannose:galactose ~ 2:1) [47].

*Odoribacter, Butyricimonas, Faecalibacterium, Lachnospira, Turicibacter, Bilophila* and *Akkermansia* populations all significantly decreased after 24 h of exposure to PHGG ($p < 0.05$). *Parabacteroides* and *Phascolarctobacterium* populations significantly increased after exposure to PHGG ($p < 0.05$). These shifts in targeted genera allowed for a significant change in the ratio of Bacteroidetes:Firmicutes. The overall abundance of Firmicutes decreased from 44.87% of sequence reads at baseline to 40.43% of sequence reads after 24 h ($p = 0.0527$). Bacteroidetes increased from 44.89% of sequence reads at baseline to 50.29% of sequence reads after 24 h ($p = 0.0008$).

Various mouse models have shown lean mice to have increased

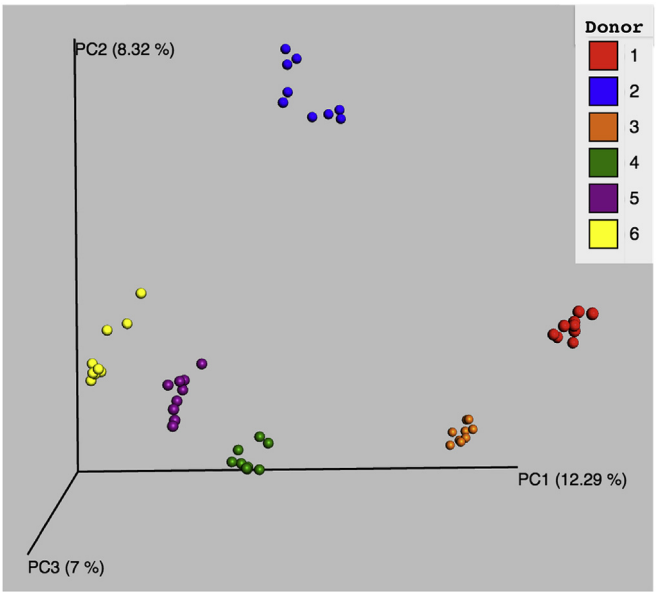


**Fig. 4.** Principal coordinates analysis (PCoA) of analyzed bacterial composition of donor samples, including 0, 12 and 24 h samples for all donors. Identifications displayed as Unweighted Unifrac distances among all samples for respective donors displayed based on their generated 99% OTU composition.

ratios of Bacteroidetes:Firmicutes compared to obese mice [48,49]. In human intervention studies, similar increased ratios of Bacteroidetes:Firmicutes have been seen following weight loss [50], although have been contradicted elsewhere [51]. A recent study has also shown increased proportions of Bacteroidetes:Firmicutes ratios with fiber supplementation, independent of caloric restriction, which was associated with total fiber intake and not BMI [52]. PHGG, along with many other types of dietary fiber, may offer feasible ways to increase Bacteroidetes in healthy individuals, although the precise function of these bacteria still remains largely unclear.

In conclusion, PHGG offers similar bacterial stimulation as other dietary fibers, most notably similar in the stimulation of *Parabacteroides* and Bacteroidetes. PHGG is a fermentable, versatile fiber that can be used in many applications as a way to help consumers bridge the gap between recommended intake and actual fiber intake, especially for consumers with IBS or related issues, as PHGG supplementation has been shown to alleviate IBS symptoms. PHGG offers stimulation of beneficial bacteria and produces significant amounts of SCFA within 24 h of exposure to microbiota, thus displaying effective prebiotic properties, and potentially therapeutic effects.

## Acknowledgements

The authors would like to thank Julian Esparza and Jonathon Swan for the laboratory assistance, the University of Minnesota Genomics Center for the guidance and analysis of the samples and The University of Minnesota Doctoral Dissertation Fellowship for support of research.

## References

[1] M. Reicks, S. Jonnalagadda, A.M. Albertson, N. Joshi, Total dietary fiber intakes in the US population are related to whole grain consumption: results from the National Health and Nutrition Examination Survey 2009 to 2010, Nutr. Res. 34 (2014) 226–234.
[2] A. Papathanasopoulos, M. Camilleri, Dietary fiber supplements: effects in obesity and metabolic syndrome and relationship to gastrointestinal functions, Gastroenterology 138 (2010) 65–72.
[3] J.L. Slavin, Dietary fiber and body weight, Nutrition 21 (2005) 411–418.
[4] M.A. Pereira, E. O'Reilly, K. Augustsson, G.E. Fraser, U. Goldbourt, B.L. Heitmann, G. Hallmans, P. Knekt, S. Liu, P. Pietinen, D. Spiegelman, J. Stevens, J. Virtamo, W.C. Willett, A. Ascherio, Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies, Arch. Intern. Med. 164 (2004) 370–376.
[5] D.J. Jenkins, C.W. Kendall, V. Vuksan, E. Vidgen, T. Parker, D. Faulkner, C.C. Mehling, M. Garsetti, G. Testolin, S.C. Cunnane, M.A. Ryan, P.N. Corey, Soluble fiber intake at a dose approved by the US Food and Drug Administration for a claim of health benefits: serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial, Am. J. Clin. Nutr. 75 (2002) 834–839.
[6] J.W. Anderson, D.A. Deakins, T.L. Floore, B.M. Smith, S.E. Whitis, Dietary fiber and coronary heart disease, Crit. Rev. Food Sci. Nutr. 29 (2009) 95–147.
[7] M. Roberfroid, Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects, Crit. Rev. Food Sci. Nutr. 33 (1993) 103–148.
[8] J. Slavin, Fiber and prebiotics: mechanisms and health benefits, Nutrients 5 (2013) 1417–1435.
[9] R. Jumpertz, D.S. Le, P.J. Turnbaugh, C. Trinidad, C. Bogardus, J.I. Gordon, J. Krakoff, Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans, Am. J. Clin. Nutr. 94 (2011) 58–65.
[10] J.R. Goldsmith, R.B. Sartor, The role of diet on intestinal microbiota metabolism: downstream impacts on host immune function and health, and therapeutic implications, J. Gastroenterol. 49 (2014) 785–798.
[11] J.K. Nicholson, E. Holmes, J. Kinross, R. Burcelin, G. Gibson, W. Jia, S. Pettersson, Metabolic interactions, Science 108 (2012) 1262–1268 (80-. ).
[12] H. Tilg, A. Kaser, Gut microbiome, obesity, and metabolic dysfunction, J. Clin. Invest. 121 (2011) 2126–2132.
[13] C.T. Brown, A.G. Davis-Richardson, A. Giongo, K.A. Gano, D.B. Crabb, N. Mukherjee, G. Casella, J.C. Drew, J. Ilonen, M. Knip, H. Hyöty, R. Veijola, T. Simell, O. Simell, J. Neu, C.H. Wasserfall, D. Schatz, M.A. Atkinson, E.W. Triplett, Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes, PLoS One 6 (2011) 1–9.
[14] N. Larsen, F.K. Vogensen, F.W.J. Van Den Berg, D.S. Nielsen, A.S. Andreasen, B.K. Pedersen, W.A. Al-Soud, S.J. Sørensen, L.H. Hansen, M. Jakobsen, Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults, PLoS One 5 (2010) 9085–9086.
[15] D.M. Saulnier, K. Riehle, T.A. Mistretta, M.A. Diaz, D. Mandal, S. Raza, E.M. Weidler, X. Qin, C. Coarfa, A. Milosavljevic, J.F. Petrosino, S. Highlander, R. Gibbs, S.V. Lynch, R.J. Shulman, J. Versalovic, Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome, Gastroenterology 141 (2011) 1782–1791.
[16] E.R. Mardis, The impact of next-generation sequencing technology on genetics, Trends Genet. 24 (2008) 133–141.
[17] M.J. Claesson, Q. Wang, O. O'Sullivan, R. Greene-Diniz, J.R. Cole, R.P. Ross, P.W. O'Toole, Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions, Nucleic Acids Res. 38 (2010).
[18] E.G. Giannini, C. Mansi, P. Dulbecco, V. Savarino, Role of partially hydrolyzed guar gum in the treatment of irritable bowel syndrome, Nutrition 22 (2006) 334–342.
[19] J.H. Lavin, N.W. Read, The effect on hunger and satiety of slowing the absorption of glucose: relationship with gastric emptying and postprandial blood glucose and insulin responses, Appetite 25 (1995) 89–96.
[20] E.M. Kovacs, M.S. Westerterp-Plantenga, W.H. Saris, I. Goossens, P. Geurten, F. Brouns, The effect of addition of modified guar gum to a low-energy semisolid meal on appetite and body weight loss, Int. J. Obes. Relat. Metab. Disord. 25 (2001) 307–315.
[21] K.J. Koecher, J.A. Noack, D.A. Timm, A.S. Klosterbuer, W. Thomas, J.L. Slavin, Estimation and interpretation of fermentation in the gut: coupling results from a 24 h batch in vitro system with fecal measurements from a human intervention feeding study using fructo-oligosaccharides, inulin, gum acacia, and pea fiber, J. Agric. Food Chem. 62 (2014) 1332–1337.
[22] J. Carlson, A. Hospattankar, P. Deng, K. Swanson, J. Slavin, Prebiotic Effects and fermentation kinetics of wheat dextrin and partially hydrolyzed guar gum in an in vitro batch fermentation system, Foods 4 (2015) 349–358.
[23] J. Carlson, J. Esparza, J. Swan, D. Taussig, J. Combs, J. Slavin, In vitro analysis of partially hydrolyzed guar gum fermentation differences between six individuals, Food Funct. 7 (2016) 1833–1838.
[24] J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Peña, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, QIIME allows analysis of high-throughput community sequencing data, Nat. Methods 7 (2010) 335–336.
[25] J. Garbe, T. Gould, D. Gohl, D. Knights, K. Beckman, Gopher-Pipelines Version 1, 2016.
[26] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120.
[27] A.P. Masella, A.K. Bartram, J.M. Truszkowski, D.G. Brown, J.D. Neufeld, PANDAseq: paired-end assembler for illumina sequences, BMC Bioinforma. 13

(2012) 31.
[28] M. Kverka, Z. Zakostelska, K. Klimesova, D. Sokol, T. Hudcovic, T. Hrncir, P. Rossmann, J. Mrazek, J. Kopecny, E.F. Verdu, H. Tlaskalova-Hogenova, Oral administration of Parabacteroides distasonis antigens attenuates experimental murine colitis through modulation of immunity and microbiota composition, Clin. Exp. Immunol. 163 (2011) 250–259.
[29] A. Hakansson, G. Molin, Gut microbiota and inflammation, Nutrients 3 (2011) 637–687.
[30] S.O. Noor, K. Ridgway, L. Scovell, E.K. Kemsley, E.K. Lund, C. Jamieson, I.T. Johnson, A. Narbad, Ulcerative colitis and irritable bowel patients exhibit distinct abnormalities of the gut microbiota, BMC Gastroenterol. 10 (2010) 1–9.
[31] M. Sakamoto, Y. Benno, Reclassification of Bacteroides distasonis, Bacteroides goldsteinii and Bacteroides merdae as Parabacteroides distasonis gen. nov., comb. nov., Parabacteroides goldsteinii comb. nov. and Parabacteroides merdae comb. nov, Int. J. Syst. Evol. Microbiol. 56 (2006) 1599–1605.
[32] E.G. Giannini, C. Mansi, P. Dulbecco, V. Savarino, Role of partially hydrolyzed guar gum in the treatment of irritable bowel syndrome, Nutrition 22 (2006) 334–342.
[33] J.L. Slavin, N.A. Greenberg, Partially hydrolyzed guar gum, Nutrition 19 (2003) 549–552.
[34] K. Tuohy, S. Kolida, The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides–a human volunteer study, Br. J. Nutr. 86 (2001) 341–348.
[35] T. Okubo, N. Ishihara, Effects of partially hydrolyzed guar gum intake on human intestinal microflora and its metabolism, Biosci. Biotechnol. Biochem. 58 (1994) 1364–1369.
[36] M.J. Claesson, S. Cusack, O. O'Sullivan, R. Greene-Diniz, H. de Weerd, E. Flannery, J.R. Marchesi, D. Falush, T. Dinan, G. Fitzgerald, C. Stanton, D. van Sinderen, M. O'Connor, N. Harnedy, K. O'Connor, C. Henry, D. O'Mahony, A.P. Fitzgerald, F. Shanahan, C. Twomey, C. Hill, R.P. Ross, P.W. O'Toole, Composition, variability, and temporal stability of the intestinal microbiota of the elderly, Proc. Natl. Acad. Sci. U. S. A. 108 (Suppl) (2011) 4586–4591.
[37] K.M. Maslowski, C.R. Mackay, Diet, gut microbiota and immune responses, Nat. Immunol. 12 (2011) 5–9.
[38] K.M. Maslowski, A.T. Vieira, A. Ng, J. Kranich, F. Sierro, D. Yu, H.C. Schilter, M.S. Rolph, F. Mackay, D. Artis, R.J. Xavier, M.M. Teixeira, C.R. Mackay, Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43, Nature 461 (2009) 1282–1286.
[39] T. Yatsunenko, F.E. Rey, M.J. Manary, I. Trehan, M.G. Dominguez-Bello, M. Contreras, M. Magris, G. Hidalgo, R.N. Baldassano, A.P. Anokhin, A.C. Heath, B. Warner, J. Reeder, J. Kuczynski, J.G. Caporaso, C.A. Lozupone, C. Lauber, J.C. Clemente, D. Knights, R. Knight, J.I. Gordon, Human gut microbiome viewed across age and geography, Nature 486 (2012) 222–227.
[40] J. Tap, S. Mondot, F. Levenez, E. Pelletier, C. Caron, J.-P. Furet, E. Ugarte, R. Muñoz-Tamayo, D.L.E. Paslier, R. Nalin, J. Dore, M. Leclerc, Towards the human intestinal microbiota phylogenetic core, Environ. Microbiol. 11 (2009) 2574–2584.
[41] M.A. Hall, C.B. Cole, S.L. Smith, R. Fuller, C.J. Rolles, Factors influencing the presence of faecal lactobacilli in early infancy, Arch. Dis. Child. 65 (1990) 185–188.
[42] M.J. Hopkins, G.T. Macfarlane, E. Furrie, A. Fite, S. Macfarlane, Characterisation of intestinal bacteria in infant stools using real-time PCR and northern hybridisation analyses, FEMS Microbiol. Ecol. 54 (2005) 77–85.
[43] J.E. Hill, W.M.U. Fernando, G.A. Zello, R.T. Tyler, W.J. Dahl, A.G. Van Kessel, Improvement of the representation of bifidobacteria in fecal microbiota metagenomic libraries by application of the cpn60 universal primer cocktail, Appl. Environ. Microbiol. 76 (2010) 4550–4552.
[44] J.K. Palmer, Degradation of polysaccharides bacterial, 1978, pp. 31–33.
[45] J.R. Balascio, J.K. Palmer, A.A. Salyers, Degradation of guar gum by enzymes produced by a bacterium from the human colon, J. Food Biochem. 5 (1981) 271–282.
[46] J. Tomlin, N.W. Read, C. a Edwards, B.I. Duerden, The degradation of guar gum by a faecal incubation system, Br. J. Nutr. 55 (1986) 481–486.
[47] J.L. Doublier, B. Launay, Rheology of galactomannan solutions: comparative study of guar gum and locust bean gum, J. Texture Stud. 12 (1981) 151–172.
[48] P.J. Turnbaugh, R.E. Ley, M.A. Mahowald, V. Magrini, E.R. Mardis, J.I. Gordon, An obesity-associated gut microbiome with increased capacity for energy harvest, Nature 444 (2006) 1027–1031.
[49] R.E. Ley, F. Bäckhed, P. Turnbaugh, C.A. Lozupone, R.D. Knight, J.I. Gordon, Obesity alters gut microbial ecology, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 11070–11075.
[50] R.E. Ley, P.J. Turnbaugh, S. Klein, J.I. Gordon, Microbial ecology: human gut microbes associated with obesity, Nature 444 (2006) 1022–1023.
[51] S.H. Duncan, G.E. Lobley, G. Holtrop, J. Ince, A.M. Johnstone, P. Louis, H.J. Flint, Human colonic microbiota associated with diet, obesity and weight loss, Int. J. Obes. (Lond). 32 (2008) 1720–1724.
[52] H.D. Holscher, J.G. Caporaso, S. Hooda, J.M. Brulc, G.C.J. Fahey, K.S. Swanson, Fiber supplementation influences phylogenetic structure and functional capacity of the human intestinal microbiome: follow-up of a randomized controlled trial, Am. J. Clin. Nutr. 101 (2015) 55–64.