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Article

Prebiotic Dietary Fiber and Gut Health: Comparing the In Vitro Fermentations of Beta-Glucan, Inulin and Xylooligosaccharide.

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Abstract: Prebiotic dietary fiber supplements are commonly consumed to help meet fiber recommendations and improve gastrointestinal health by stimulating beneficial bacteria and the production of short-chain fatty acids (SCFAs), molecules beneficial to host health. The objective of this research project was to compare potential prebiotic effects and fermentability of five commonly consumed fibers using an in vitro fermentation system measuring changes in fecal microbiota, total gas production and formation of common SCFAs. Fecal donations were collected from three healthy volunteers. Materials analyzed included: pure beta-glucan, Oatwell (commercially available oat-bran containing 22% oat β-glucan), xylooligosaccharides (XOS), WholeFiber (dried chicory root containing inulin, pectin, and hemi/celluloses), and pure inulin. Oatwell had the highest production of propionate at 12 h (4.76 μmol/mL) compared to inulin, WholeFiber and XOS samples (p<0.03). Oatwell's effect was similar to those of the pure beta-glucan samples, both samples promoted the highest mean propionate production at 24 h. XOS resulted in a significant increase in the genus *Bifidobacterium* after 24 h of fermentation (0 h: 0.67 OTUs; 24 h: 5.22 OTUs; p = 0.038). Inulin and WholeFiber increased the beneficial genus *Collinsella*, consistent with findings in clinical studies. All analyzed compounds were fermentable and promoted the formation of beneficial SCFAs.

Keywords: prebiotic, microbiota, fermentation, dietary fiber, microbiome

1. Introduction

Prebiotic definitions vary among different scientific and political arenas across the world [1]. Depending on the local definition, nearly all prebiotics can be classified as dietary fiber, but not all fibers are considered prebiotics [2]. The most recent definition describes a prebiotic as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" [3]. Functional characteristics of prebiotics include the ability to: resist the low pH of the stomach, resist hydrolysis by mammalian enzymes, resist absorption in the upper gastrointestinal tract, the ability to be fermented by intestinal microbiota and selectively stimulate the growth and/or activity of intestinal bacteria associated with host health and overall well-being [4,5]. Inulin, beta-glucans, and xylooligosaccharides all provide health benefits to consumers that are related to the fermentation of these compounds in the distal gastrointestinal tract, and are also considered functional fibers with many other benefits [6]. As the definition of "prebiotic" broadens to include the overall impact from the metabolism from these compounds, the category of prebiotics will expand [7]. The importance of displaying direct health benefits due to bacterial fermentation is still the driving mechanism for all prebiotics.

As our awareness and understanding of the importance of the gut microbiome and gut microbiota increases, it is imperative for consumers to understand the key differences between different forms of prebiotics, and where they can be found in various foods and food products. XOS is an emerging prebiotic with well-displayed, consistent health benefits[8] and is composed of sugar oligomers composed of xylose units [9], found naturally in fruits, vegetables, milk, honey and bamboo shoots. XOS is commonly produced from xylan containing lignocellulosic materials through various chemical methods, direct enzymatic hydrolysis, or a combination of both treatments [10–14]. Inulin is a heterogeneous blend of fructose polymers (DP<10)[15] which occurs naturally in thousands of plant species, including wheat, onion, bananas, garlic and chicory [16]. Beta-glucan is a polysaccharide composed of D-glucose monomers with beta-glycosidic linkages, present in either linear chains in grains, such as oat and barley (up to 7%), or in branched structures in fungi, yeast and certain bacteria [17]. These prebiotics, or prebiotic mixtures, each provide a unique carbon source for selective stimulation of different bacterial taxa and are important microbiota-shaping compounds.

Because no analytical method currently exists to measure the prebiotic capacity of foods in terms of their influence on gastrointestinal taxa, this field relies heavily on fecalbiotics (living or once living fecal microbial populations) to quantify the effects of these compounds. In vitro fermentation models allow for quantitative analysis of specific materials and are semi-representative models of colonic fermentation [18]. Although not a complete substitute for human studies, when paired with in vivo models, in vitro analysis can be an accurate systematic approach to analyze different parameters and end points in colonic fermentation [19].

With the recent release of the International Scientific Association for Probiotics and Prebiotics consensus statement, prebiotics have now been defined to include XOS, not previously included [20]. This paper compares the fermentation effects of XOS to previously established prebiotics (inulin and beta-glucans) in a controlled in-vitro model. To authors' knowledge this is the first controlled in vitro study comparing the effects of XOS to these known prebiotics. The objective of this project was to compare currently available prebiotics by their ability to change specific taxa as well as compare differences in the production of gas and common short chain fatty acids (SCFA) between these products. Inulin, XOS and beta-glucan based products were chosen for this experiment because they are established and emerging prebiotics that are commonly consumed, and offer well-demonstrated health benefits to their consumers.

2. Materials and Methods

2.1 Prebiotic Dietary Fibers Analyzed

Five common prebiotic dietary fibers were chosen for this study (Table 1), including different types of beta-glucans, inulin and xylooligosaccharide supplements.

Table 1. Comparison Prebiotic Dietary Fibers Analyzed With In Vitro Fermentation System.

Prebiotic Dietary Fibers	Supplier Information
OatWell (Oatbran containing 28% beta-glucan)	DSM Nutritional Products, Ltd.
WholeFiber (A dried chicory root blend containing: inulin, pectin, hemi/cellulose)	WholeFiber, Inc.
Xylooligosaccharide (XOS)	AIDP, Inc.
Pure Inulin	Cargill, Inc.
Pure Beta-glucan	Megazyme, Inc.

2.2 Fecal Collection & Donor Information

Fecal samples were collected from three healthy volunteers (2 males, 1 female) under anaerobic conditions from individuals (ages 22-28) consuming non-specific Western diets, free of any antibiotic treatments in the last year, non-smokers, not affected by any known GI diseases and not consuming any supplements (Table 2). Fecal samples were anaerobically collected within 5 minutes of the start of the fermentation (Medline Specimen Collection Kit, Medline, Inc.), and homogenized immediately upon collection. All data and samples collected were done in accordance with University of Minnesota policies and procedures.

Table 2. Demographic Characteristics of Three Fecal Donors.

	Donor 1	Donor 2	Donor 3
Age	26	25	22
Sex	Female	Male	Male
BMI	28.1	26.3	23.0

2.3 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 hours at 4°C. Following incubation, serum bottles were transferred to a circulating water bath at 37°C and allowed to incubate for 2 hours. 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 1N NaOH, 2.56 g sodium sulfide nonanhydride, 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.

2.4 SCFA Analysis

SCFA samples were extracted according to Schneider et al[21] with minor modifications, and analyzed with previously described methods [22].

2.5 DNA Extractions

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

2.5.1 Primary/Secondary 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 5x 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 1000x 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 minutes, followed by 20 cycles of

98°C for 20 seconds, 55°C for 15 seconds, and 72°C for 1 minute. 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 (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAGTTTGATCMTGGCTCAG) and Meta_V3_534R (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATTACCGCGGCTGCTGG).

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 5x 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 minutes, followed by 10 cycles of 98°C for 20 seconds, 55°C for 15 seconds, 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes. The following indexing primers were used (X indicates the positions of the 8 bp indices): Forward indexing primer: AATGATACGGCGACCACCGAGATCTACACXXXXXXXXTCGTCGGCAGCGTC and Reverse indexing primer: CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTCTCGTGGGCTCGG

2.5.2 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 2nM. 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 minutes, and sequenced using a MiSeq 600 cycle v3 kit (Illumina, San Diego, CA).

2.5.3 Sequence Processing and Analysis

Generated sequence data was processed and analyzed using QIIME [23]. Fastq sequence data was processed with the University of Minnesota's gopher-pipeline for metagenomics [24]. Sequence data had adapters removed and sliding quality trimming window by Trimmomatic [25]; primers removed and overlapping reads merged by Pandaseq [26]. 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).

2.6 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 abundancies for a given OTU is the same. Multiple comparisons were corrected using the FDR procedure. For gas and SCFA data, ANOVA with Tukey HSD was used to compare means. Significance was set for p-values < 0.05 for all statistical tests.

2.7 Consent

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

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3. Results

3.1 Gas Production

At 12 h, the OatWell and the pure beta-glucan samples produced similar amounts of total gas (Figure 1). The XOS samples produced significantly more gas than the pure beta glucan samples ($p < 0.01$) or the OatWell samples ($p < 0.01$). The WholeFiber and pure inulin samples produced similar amounts of total gas ($p = 0.102$), and the total gas production for both of these prebiotic dietary fibers was significantly higher than the XOS samples, ($p < 0.01$ and $p = 0.045$), respectively. At 24 h, the OatWell samples had the lowest gas production (46.2 mL) and were similar to the pure beta-glucan samples (63.7 mL; $p = 0.498$). The 24 h XOS samples (74.0 mL) were also similar to the beta-glucan samples ($p = 0.926$). However, the 24 h WholeFiber (109.6 mL) and pure inulin (107.1 mL) samples produced significantly more gas than XOS, beta-glucan and Oatwell samples ($p < 0.01$).

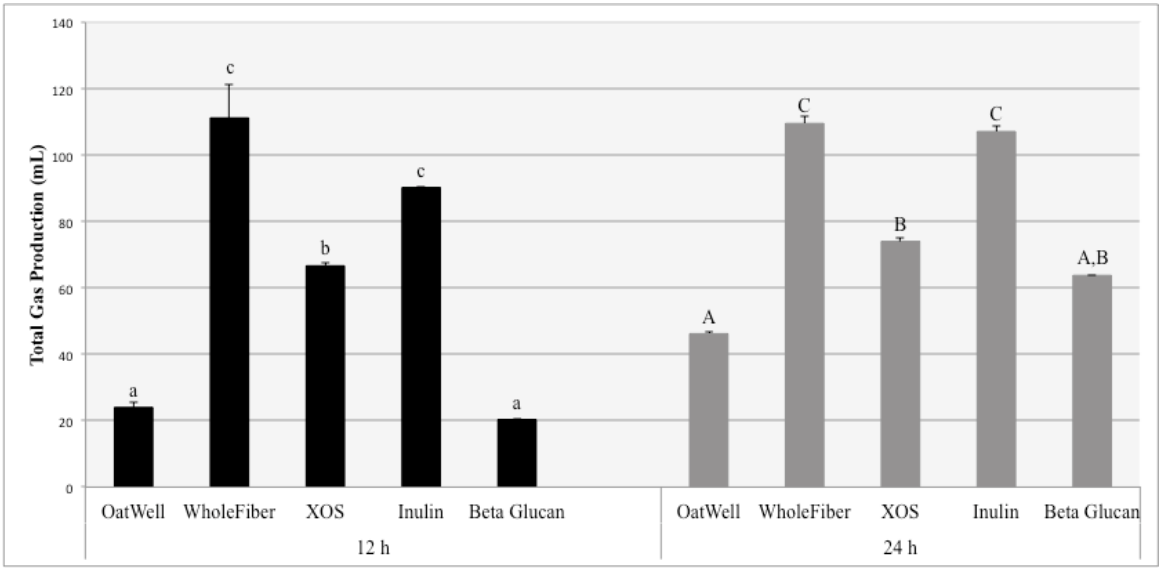

Figure 1. Total gas production comparing fermentation differences among five prebiotic dietary fibers for three individuals at 12 h and 24 h post-exposure to fecal microbiota in an in vitro fermentation system. Data displayed are means (3 donors x 3 replicates = 9) for each prebiotic dietary fiber ± SD. Columns with different letters are significantly different from one another within each time measurement (lowercase: 12 h; uppercase: 24 h). Data were analyzed using ANOVA with Tukey HSD ($p < 0.05$).

3.2 SCFA Production

For all SCFA analysis, analysis at 12 and 24 h shows production only, from baseline corrected samples. Acetate, propionate and butyrate production is shown as µmol/mL of fermentation media.

Acetate production at 12 h was similar for the Oatwell, WholeFiber and beta-glucan samples (Figure 2). The XOS samples produced significantly more acetate at 12 h than the Oatwell, WholeFiber or beta-glucan samples ($p<0.05$). The inulin samples had similar amounts of acetate compared to the WholeFiber and XOS samples, and significantly more than the Oatwell ($p=0.024$) and beta-glucan ($p=0.013$) samples at 12 h. After 24 h, the inulin samples contained less acetate than the XOS samples ($p=0.038$), while the Oatwell, WholeFiber and beta-glucan samples were similar to both the XOS and inulin samples.

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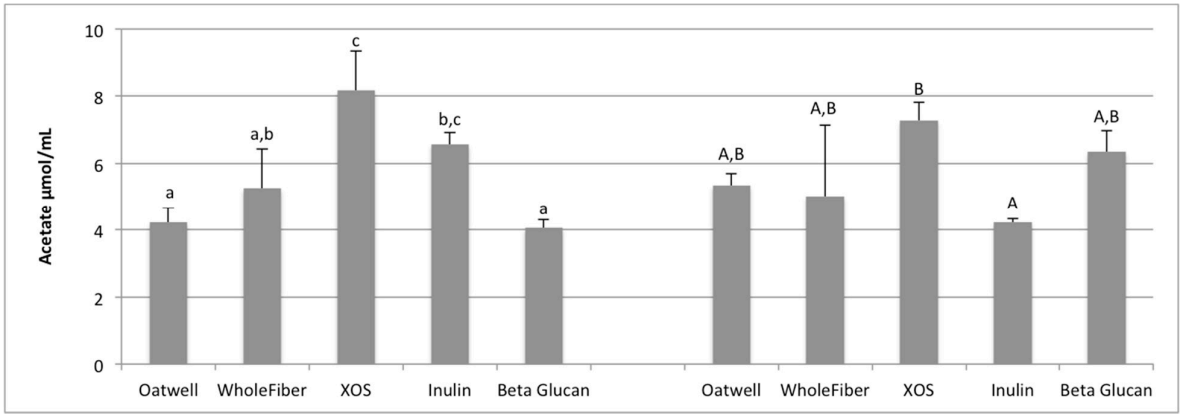

Figure 2. Acetate production at 12 h and 24 h of fermentation for five prebiotic dietary fibers displayed as µmol/mL of fermentation inoculum. Data displayed are means (3 donors x 3 replicates = 9) for each prebiotic dietary fiber ± SD. Columns with different letters are significantly different from one another (lowercase: 12 h; uppercase: 24 h). Data were analyzed using ANOVA with Tukey HSD ($p < 0.05$).

Propionate production at 12 h of fermentation was highest for the OatWell samples (4.76 µmol/mL) and was significantly greater than the WholeFiber ($p=0.029$), XOS ($p=0.005$) and inulin samples ($p=0.004$), and similar to the beta-glucan samples (Figure 3). At 24 h of fermentation, the Oatwell samples had the highest mean production 5.05 µmol/mL, which was significantly greater than the XOS samples (2.58 µmol/mL; $p=0.021$), and similar to WholeFiber, inulin and beta-glucan samples.

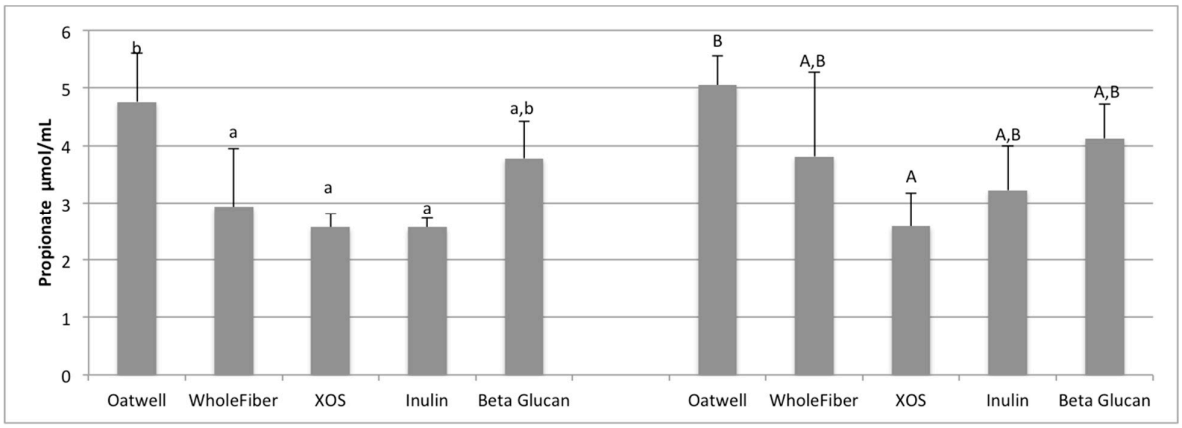

Figure 3. Propionate production at 12 h of fermentation for five prebiotic dietary fibers displayed as µmol/mL of fermentation inoculum. Data displayed are means (3 donors x 3 replicates = 9) for each prebiotic dietary fiber ± SD. Columns with different letters are significantly different from one another (lowercase: 12 h; uppercase: 24 h). Data were analyzed using ANOVA with Tukey HSD ($p < 0.05$).

Butyrate production after 12 h of fermentation ranged from 7.30 µmol/mL for the beta-glucan samples to 16.76 µmol/mL for the inulin samples (Figure 4). The inulin samples had the highest average production, and were similar to the XOS (16.38 µmol/mL) and WholeFiber samples (12.89 µmol/mL). The XOS samples were significantly higher than the Oatwell ($p=0.035$) and beta-glucan samples ($p=0.014$). At 24 h of fermentation, all five prebiotic dietary fibers were statistically similar to one another, ranging from 7.93 – 14.08 µmol/mL due to a wide ranges in response differences between the three fecal donors used in this study.

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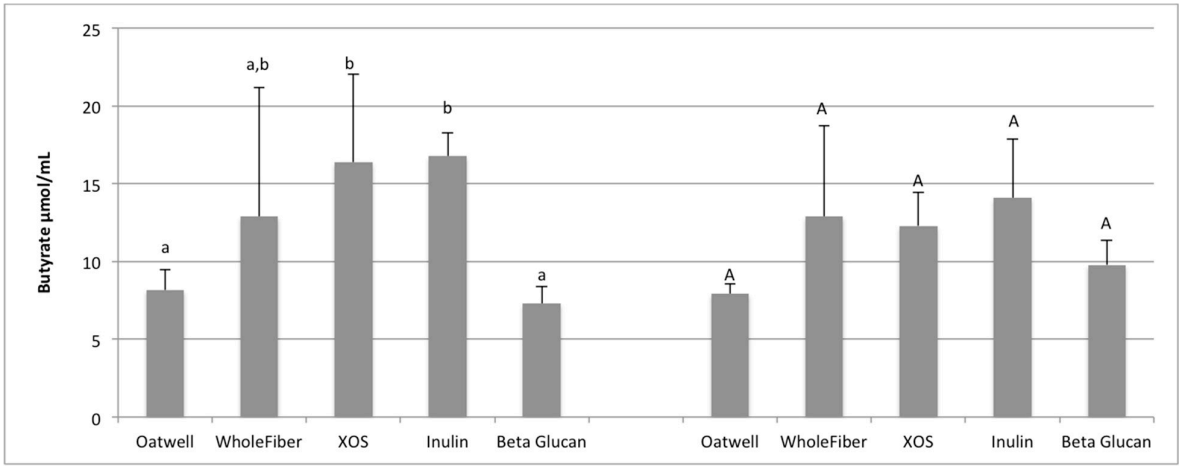

Figure 4. Butyrate production at 12 h of fermentation for five prebiotic dietary fibers displayed as µmol/mL of fermentation inoculum. Data displayed are means (3 donors x 3 replicates = 9) for each prebiotic dietary fiber ± SD. Columns with different letters are significantly different from one another (lowercase: 24 h; uppercase: 24 h). Data were analyzed using ANOVA with Tukey HSD ($p < 0.05$).

3.3 Microbiota Analysis

Extracted DNA from in vitro samples were sequenced using the MiSeq Illumina platforms, generating a total of 31,591,899 sequence reads. Sequencing parameters identified reads belonging to 11 bacterial phyla, 61 families and 97 genera.

For all three donors, the phyla Bacteroidetes and Firmicutes represented >80% of all sequence reads (Figure S1) across 24 h of fermentation. At the family level, 13 families represented 85% of all sequence reads (Figure S2), while 11 genera represented >75% of all sequence reads (Figure S3). Six metrics measuring α-diversity for all donors showed various degrees of grouping by donors (Figure S4), and by treatment group (Figure S5). Both Unifrac and Bray-Curtis β-diversity metrics (measuring pairwise dissimilarity between samples), showed similarity among technical replicates of treatment groups for each donor (Figure S6) as well as for all treatment groups for each respective donor (Figure S7).

After 24 h of fermentation, the Oatwell samples significantly decreased the population of SMB53 (0 h: 9.11 OTUs; 24 h: 2.11 OTUs; $p = 0.008$), *Lachnospira* and *Faecalibacterium* (0 h: 26.56 OTUs; 24 h: 4.44 OTUs; $p = 0.008$ and 0 h 136.44 OTUs; 24 h: 66 OTUs; $p = 0.022$, respectively) (Table 3). In vivo studies with inulin, scFOS and resistant starch supplementation have all found decreases in the SMB53 genus [27,28]. No genera analyzed showed significant increases in 24 h for the Oatwell samples measured for the three fecal donors in this study. The WholeFiber samples (Table 4) significantly increased the genus *Collinsella* at 24 h compared to 0 h (0 h: 68 OTUs; 24 h: 299.78 OTUs; $p = 0.011$). *Bifidobacterium* populations were only significantly increased at 24 h compared to 0 h for the XOS samples (0 h: 0.67 OTUs; 24 h: 5.22 OTUs; $p = 0.038$), while the same samples showed a significant decrease in *Lachnospira* and *Faecalibacterium* ($p = 0.038$ and $p = 0.03$) (Table 5). The inulin samples (Table 6) increased *Collinsella* (0 h: 55.11 OTUs; 24 h: 291.44 OTUs; $p = 0.016$). The pure beta glucan samples significantly decreased *Lachnospira* and *Faecalibacterium* ($p = 0.008$) (Figure 7).

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Table 3. Combined changes across 24 h of fermentation for Oatwell samples of identified phyla and genera.[1]

Phyla & Genera	0 h	24 h	Test-Statistic	P-value
Actinobacteria				
Bifidobacterium	1.22	0.89	0.63	0.660
Adlercreutzia	1.44	3.00	1.37	0.470
Collinsella	48.44	140.56	6.33	0.089
Bacteroidetes				
Alistipes	2.56	1.33	0.55	0.674
Parabacteroides	135.00	155.89	0.05	0.952
Bacteroides	755.67	865.00	3.95	0.192
Firmicutes				
Eubacterium	0.44	0.56	0.20	0.817
Veillonella	1.11	1.22	0.01	0.980
Dorea	2.33	3.56	1.08	0.516
Acidaminococcus	3.22	10.44	0.22	0.817
Clostridium	7.67	8.33	0.33	0.769
Anaerostipes	8.11	6.00	0.52	0.674
Turicibacter	8.67	1.22	2.54	0.286
SMB53	9.11	2.11	12.83	0.008*
Ruminococcus	11.22	23.22	3.13	0.263
Lactococcus	11.67	10.67	0.00	0.980
Streptococcus	15.22	8.11	1.15	0.511
Roseburia	20.22	22.33	0.01	0.980
Oscillospira	21.78	36.67	4.90	0.121
Lachnospira	26.56	4.44	12.94	0.008*
Phascolarctobacterium	27.78	173.33	3.03	0.263
Dialister	39.56	43.00	0.88	0.560
Blautia	41.89	53.11	1.32	0.470
Coprococcus	49.89	39.00	1.76	0.396
Ruminococcus	61.33	40.67	2.39	0.289
Faecalibacterium	136.44	66.00	9.58	0.022*
Proteobacteria				
Escherichia	0.44	1.44	2.13	0.325
Haemophilus	10.22	0.67	2.49	0.286
Sutterella	10.78	14.44	0.00	0.980
Bilophila	13.67	14.78	0.28	0.788
Verrucomicrobia				
Akkermansia	5.00	12.00	0.01	0.980

[1] Replicate samples were pooled among donor at each respective time point (3 donors x 3 replicated = 9). Samples were analyzed between differentially represented OTUs for significant changes after 24

h of fermentation compared to 0 h samples. Data were analyzed using the Kruskal-Wallis ANOVA test, with the FDR multiple comparisons correction. *Indicates significance at P≤ 0.05.

Table 4. Combined changes across 24 h of fermentation for WholeFiber samples of identified phyla and genera.[1]

Phyla & Genera	0 h	24 h	Test-Statistic	P-value
Actinobacteria				
Adlercreutzia	0.89	3.89	3.61	0.239
Bifidobacterium	1.11	1.11	0.56	0.785
Collinsella	68.00	299.78	12.17	0.011*
Bacteroidetes				
Alistipes	1.11	0.56	0.31	0.894
Parabacteroides	131.44	142.00	0.07	0.913
Bacteroides	743.56	776.56	0.56	0.785
Firmicutes				
Eubacterium	1.11	0.78	0.50	0.799
Veillonella	1.22	1.00	0.01	0.960
Dorea	2.00	5.00	0.66	0.785
Acidaminococcus	2.67	11.33	0.22	0.894
SMB53	5.67	4.00	3.58	0.239
Clostridium	7.33	13.22	0.10	0.896
Anaerostipes	10.22	1.22	3.60	0.239
Ruminococcus	10.89	19.67	0.03	0.943
Streptococcus	12.67	8.78	0.57	0.785
Turicibacter	14.22	2.44	0.01	0.960
Lachnospira	14.78	72.00	4.13	0.237
Oscillospira	17.22	14.89	1.13	0.647
Lactococcus	20.44	9.22	0.12	0.896
Phascolarctobacterium	24.67	60.44	1.82	0.501
Dialister	26.11	58.22	0.03	0.943
Roseburia	28.56	6.00	0.95	0.674
Blautia	32.44	49.44	5.08	0.156
Coprococcus	45.78	66.44	1.64	0.501
Ruminococcus	54.22	39.33	3.29	0.261
Faecalibacterium	154.89	93.11	7.75	0.080
Proteobacteria				
Escherichia	0.78	1.44	0.00	0.960
Sutterella	4.00	32.44	0.22	0.894
Haemophilus	10.67	0.56	6.72	0.107
Bilophila	10.67	7.67	0.10	0.896
Verrucomicrobia				
Akkermansia	17.00	3.33	1.67	0.501

[1] Replicate samples were pooled among donor at each respective time point (3 donors x 3 replicated = 9). Samples were analyzed between differentially represented OTUs for significant changes after 24 h of fermentation compared to 0 h samples. Data were analyzed using the Kruskal-Wallis ANOVA test, with the FDR multiple comparisons correction. * Indicates significance at P≤ 0.05.

Table 5. Combined changes across 24 h of fermentation for xylooligosaccharide samples of identified phyla and genera.[1]

Phyla & Genera	0 h	24 h	Test-Statistic	P-value
Actinobacteria				
Bifidobacterium	0.67	5.22	9.11	0.038*
Adlercreutzia	1.33	1.78	0.08	0.972
Collinsella	58.44	154.00	1.87	0.413
Bacteroidetes				
Alistipes	1.44	0.56	1.85	0.413
Parabacteroides	147.33	133.33	0.02	0.972
Bacteroides	770.89	870.44	4.31	0.189
Firmicutes				
Eubacterium	0.33	1.67	2.75	0.364
Veillonella	0.67	0.00	4.78	0.162
Acidaminococcus	1.33	2.33	0.03	0.972
Dorea	2.11	3.67	1.70	0.423
SMB53	7.33	5.44	1.14	0.558
Anaerostipes	7.44	3.44	1.51	0.447
Turicibacter	8.00	8.56	0.02	0.972
Clostridium	8.44	4.00	6.51	0.087
Ruminococcus	12.78	26.11	6.58	0.087
Streptococcus	14.11	4.67	2.46	0.367
Lachnospira	21.11	5.33	9.37	0.038*
Oscillospira	21.33	21.78	0.02	0.972
Phascolarctobacterium	23.44	16.33	0.10	0.972
Lactococcus	23.89	21.00	0.00	0.982
Roseburia	28.89	35.33	0.16	0.972
Dialister	33.89	41.56	0.42	0.831
Blautia	39.22	65.00	6.37	0.087
Ruminococcus	45.11	37.33	2.13	0.385
Coprococcus	47.11	48.67	0.78	0.705
Faecalibacterium	148.56	79.56	11.56	0.030*
Proteobacteria				
Escherichia	0.89	0.44	0.07	0.972
Haemophilus	6.44	3.11	0.03	0.972
Bilophila	17.78	6.22	5.73	0.107
Sutterella	25.78	40.89	0.43	0.831
Verrucomicrobia				
Akkermansia	2.78	5.00	0.34	0.841

[1] Replicate samples were pooled among donor at each respective time point (3 donors x 3 replicated = 9). Samples were analyzed between differentially represented OTUs for significant changes after 24 h of fermentation compared to 0 h samples. Data were analyzed using the Kruskal-Wallis ANOVA test, with the FDR multiple comparisons correction. * Indicates significance at $P \leq 0.05$.

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Table 6. Combined changes across 24 h of fermentation for pure inulin samples of identified phyla and genera.[1]

Phyla & Genera	0 h	24 h	Test-Statistic	P-value
Actinobacteria				
Bifidobacterium	1.33	5.44	3.94	0.304
Adlercreutzia	1.33	2.00	0.47	0.845
Collinsella	55.11	291.44	12.79	0.016*
Bacteroidetes				
Alistipes	1.56	0.89	0.36	0.878
Parabacteroides	147.44	164.78	0.28	0.887
Bacteroides	726.78	644.44	1.64	0.652
Firmicutes				
Veillonella	0.78	0.56	0.03	0.908
Eubacterium	0.89	1.56	0.02	0.908
Dorea	1.78	7.00	1.96	0.640
Acidaminococcus	3.11	18.67	0.22	0.887
SMB53	7.44	9.11	0.00	0.965
Turicibacter	7.78	4.89	1.06	0.652
Clostridium	8.22	7.11	0.51	0.845
Ruminococcus	9.56	34.11	3.61	0.309
Anaerostipes	11.22	4.67	1.17	0.652
Streptococcus	13.00	12.44	0.16	0.887
Lactococcus	19.11	9.67	0.02	0.908
Lachnospira	21.00	4.89	10.85	0.022*
Phascolarctobacterium	26.22	21.00	0.16	0.887
Oscillospira	26.33	10.11	9.30	0.034*
Roseburia	26.78	14.11	0.28	0.887
Dialister	32.67	95.11	0.16	0.887
Blautia	38.22	50.22	0.86	0.690
Coprococcus	48.11	60.89	1.88	0.640
Ruminococcus	52.33	43.00	0.10	0.908
Faecalibacterium	148.11	187.33	1.22	0.652
Proteobacteria				
Escherichia	1.00	1.22	0.04	0.908
Haemophilus	9.11	2.67	1.45	0.652
Sutterella	14.00	31.22	0.02	0.908
Bilophila	16.89	7.78	3.31	0.309
Akkermansia	7.78	7.44	4.02	0.304

[1] Replicate samples were pooled among donor at each respective time point (3 donors x 3 replicated = 9). Samples were analyzed between differentially represented OTUs for significant changes after 24 h of fermentation compared to 0 h samples. Data were analyzed using the Kruskal-Wallis ANOVA test, with the FDR multiple comparisons correction. * Indicates significance at $P \leq 0.05$.

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Table 7. Combined changes across 24 h of fermentation for pure beta-glucan samples of identified phyla and genera.[1]

Phyla & Genera	0 h	24 h	Test-Statistic	P-value
Actinobacteria				
Bifidobacterium	0.33	0.33	0.00	1.000
Adlercreutzia	2.00	1.89	0.07	0.843
Collinsella	69.22	85.11	0.86	0.723
Bacteroidetes				
Alistipes	0.78	0.89	0.32	0.778
Parabacteroides	119.56	179.78	0.56	0.778
Bacteroides	776.11	854.33	1.03	0.664
Firmicutes				
Eubacterium	0.11	0.44	0.46	0.778
Veillonella	0.56	0.22	0.38	0.778
Dorea	0.89	3.11	7.06	0.110
Acidaminococcus	2.33	15.11	0.22	0.778
SMB53	6.11	4.89	0.39	0.778
Lactococcus	6.11	0.67	0.22	0.778
Anaerostipes	7.44	5.22	0.40	0.778
Turicibacter	8.11	3.00	0.13	0.803
Ruminococcus	9.44	18.67	4.35	0.166
Clostridium	10.11	3.33	6.38	0.110
Streptococcus	14.89	6.44	3.32	0.256
Roseburia	16.11	54.33	1.54	0.510
Lachnospira	21.22	3.89	12.88	0.008*
Oscillospira	24.33	35.11	2.40	0.389
Phascolarctobacterium	29.00	125.33	3.03	0.283
Dialister	30.56	43.67	0.10	0.819
Coprococcus	44.11	20.78	3.79	0.211
Blautia	45.11	68.11	2.13	0.408
Ruminococcus	59.67	44.44	1.64	0.500
Faecalibacterium	152.11	62.67	12.82	0.008*
Proteobacteria				
Escherichia	0.89	0.56	0.31	0.778
Haemophilus	11.00	0.78	5.45	0.110
Sutterella	14.00	35.44	0.22	0.778
Bilophila	14.44	13.89	0.20	0.778
Verrucomicrobia				
Akkermansia	9.11	15.89	0.45	0.778

[1] Replicate samples were pooled among donor at each respective time point (3 donors x 3 replicated = 9). Samples were analyzed between differentially represented OTUs for significant changes after 24 h of fermentation compared to 0 h samples. Data were analyzed using the Kruskal-Wallis ANOVA test, with the FDR multiple comparisons correction. * Indicates significance at $P \leq 0.05$.

4. Discussion

The aim of this study was to investigate the beneficial effects of commonly consumed prebiotic dietary fibers, including their ability to influence the growth of identified bacterial populations, form beneficial SCFAs, and the amount of gas they produce due to fermentation. Total gas production due to fiber fermentation depends on a wide range of factors. The inulin samples and the WholeFiber samples (mixture of dried chicory root inulin, pectin and hemi/cellulose) resulted in the

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highest gas production at both 12 and 24 h. These results are consistent with results from both clinical feeding studies and other in vitro experiments, in which fermentation of inulin products resulted in high amounts of gas production, sometimes resulting in mild negative GI symptoms, depending on the dosage [29,30]. Similar in vitro studies have found inulin to be much more fermentable than beta-glucan products, for both barley and oat-derived beta-glucans [31]. XOS fermentation results in less gas production than the inulin products, and more gas than beta-glucan products. Because of these findings, previous studies based on digestive tolerance and parameters have established a tolerated daily dosage of approximately 12 g/d [32].

SCFA production due to the fermentation of prebiotic dietary fibers promotes many beneficial health outcomes to the host. SCFA production may contribute to up to 10% of the host's metabolizable energy daily, with production of total SCFAs usually between 100-200 mM/d, but is highly dependent on the donor and availability of substrates for fermentation [33,34]. At 12 h of fermentation, the OatWell and beta-glucan samples had significantly higher concentrations of propionate, and the highest mean concentration at 24 h compared to the other prebiotic dietary fibers analyzed. Similar in vitro studies with beta-glucan based products have also shown similar preference for these products to result in propionate formation [31]. Although no mechanism has been identified, and studies show conflicting results results [35], elevated serum propionate concentrations have been shown to have a hypocholesterolaemic effect [36]. Propionate may also play an influential role in satiety, although mechanisms still remain unclear [37,38]. Cholesterol-lowering properties of beta-glucans may be limited to effects from the upper-GI, although many propionate-producing bacteria have a preference to fermenting various types of beta-glucans (*Bacteroides, Prevotella, Clostridium*) based on the presence of genes responsible for endo-β-glucanase enzyme production [39].

Microbial diversity among fecal donors complicates the identification of trends among the five treatment groups (Supplemental Figures 8 and 9). In terms of taxonomic shift, the inulin-based products were fermented nearly identically by all three fecal donors. Both pure inulin and WholeFiber promoted the growth of *Collinsella* comparing the 24 h samples to 0 h samples. Inulin-type fructans have been shown in clinical studies to promote substantial growth of *Collinsella*, paralleled with increased urinary hippurate levels [40]. Hippurate is a metabolite derived from various fermentation processes in the gut that has been found in decreased concentrations in obese individuals compared to lean individuals, and also between diabetics and non-diabetics [41–43]. The genus *Collinsella* has been found in lower concentrations in individuals with IBD compared to healthy controls[44], while *Collinsella aerofaciens* has been associated with low risk of colorectal cancer [45]. Increases in *Collinsella* and increased urinary hippurate levels are considered a beneficial effect of inulin consumption due to its prebiotic capacity [40].

A significant increase in the genus *Bifidobacterium* was observed only with the XOS treatment. Increases in *Bifidobacterium* have been heavily studied and reviewed, and are considered a beneficial effect due to their correlation with many positive health outcomes [1]. *Bifidobacteria* reside naturally in the gastrointestinal tract of healthy human adults and have a strong affinity to ferment oligosaccharides, making them a common marker for prebiotic capacity. *Bifidobacterium* is a unique

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329 genus of bacteria in that no gas is formed as an end product of metabolism [46]. Like *Lactobacillus*,
330 these bacteria are saccharolytic, often considered a beneficial trait [47]. *Bifidobacteria* also do not
331 produce any known carcinogenic substances in vivo. *Bifidobacteria* concentrations have been
332 negatively associated with obesity and weight gain.[48–51] Increases in *Bifidobacteria* have also been
333 correlated with a decrease in blood lipopolysaccharides (LPS), inflammatory reagents that play a
334 role in the development of inflammatory metabolic disorders and conditions, and are primarily
335 found in gram-negative bacteria [52]. LPS induce the activation of Toll-like receptor 4 (TLR4), which
336 leads to inflammation due to release of pro-inflammatory cytokines and chemokines [53].
337
338 In vitro fermentations are semi-representative models of colonic fermentation, but have
339 limitations [18]. This study did not include an in vitro digestion process, which would remove
340 digestible contents from the samples prior to fermentation, and would be a more representative
341 model. However, because the test substrates are primarily fiber, which is non-digestible, this should
342 have minimal impact on the results of this study. In vivo, formed gases are continually absorbed
343 and colonic absorption is rapid. Because SCFAs are rapidly absorbed and difficult to measure, in
344 vitro models help to understand the kinetics of colonic fermentation. However, in vitro models must
345 be paired with similar in vivo models to better understand the full mechanisms of action resulting
346 from colonic fermentation of prebiotic dietary fibers.
347

348 **5. Conclusions**

349 All five prebiotics measured in this study offer specific health benefits that can be attributed to
350 their fermentation. Depending on their structure, each compound offers a specific carbon source for
351 fermentation by different bacterial populations, yielding changes in beneficial taxa and production
352 of various amounts of SCFAs and gas in vitro. For instance, while OatWell and beta-glucans
353 promoted propionate production, XOS increased concentrations of *Bifodobacterium*, and WholeFiber
354 and pure inulin promoted *Collinsella* growth. Findings in this study are consistent to other in vitro
355 studies with similar prebiotic dietary fibers, as well as numerous clinical feeding studies.
356
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Supplementary Materials: Supplementary Materials are available online.

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