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In Vitro Effects of Dietary Inulin on Human Fecal Microbiota and Butyrate Production

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Administration of dietary fibers has various health benefits, mainly by increasing numbers of beneficial bacteria and enhancing production of short-chain fatty acids in the colon. There has been growing interest in the addition of dietary fiber to human diet, due to its prebiotic effects. This study aimed to evaluate the prebiotic activity of inulin using an *in vitro* batch fermentation system with human fecal microbiota. Fermentation of inulin resulted in a significantly greater ratio of *Lactobacillus* or *Bifidobacteria* to *Enterobacteria* strains as an index of healthy human intestine and elevated butyrate concentration, which are related to improvement of gut health.

Keywords: *In vitro* fermentation, human fecal microbiota, inulin, butyrate

Dietary fibers are reported to exhibit various health benefits, mainly from increased numbers of beneficial bacteria such as *Lactobacillus* and *Bifidobaceria*, and enhanced production of short-chain fatty acids in the colon [4, 9]. In addition, administration of dietary fibers is known to demonstrate other beneficial outcomes on hyperlipidemia, ulcerative colitis, osteoporosis, and compromised immune function [11]. There has been growing interest in the addition of different dietary fibers to human diet owing to their prebiotic effects [22]. However, it is crucial to understand the potential effects of dietary fibers before incorporating them into functional foods.

Inulin is a natural fructose polymer with a terminal glucose unit and is present in many types of plants, including wheat, onion, banana, garlic, and chicory [13]. The most well-known benefit of inulin consumption is improvement of intestinal health after reaching the colon intact [18]. In addition, it has been reported that inulin modulates immune function, and reduces the risk of colon cancer and cardiovascular disease [15, 17, 20].

This study aimed to develop an *in vitro* batch fermentation system with human fecal microbiota and to evaluate the prebiotic activity of inulin by investigating fermentation profiles such as bacterial community and fatty acid production.

Fresh feces were collected on site under anaerobic conditions from five Korean healthy male volunteers (age range 20–25 years). Feces were immediately homogenized for 5 min with an equal quantity of phosphate-buffered saline (PBS; 0.1 M, pH 7.0). Aliquots of fecal slurry filtered through cheesecloths were transferred to 50 ml McCartney bottles containing PBS alone as a negative control, cellulose (Daejung Chemicals, Korea) as a positive control, and inulin (Sigma, USA) at final concentrations of 5%. Each bottle of three treatments ($n = 5$) was flushed with CO_2 and incubated at 37°C for 24 h using a shaking incubator at 200 rpm.

Bacterial DNAs extracted from the fermented samples were applied to quantitative real-time PCR using the StepOnePlus real-time PCR system (Applied Biosystems, USA), with specific primers for individual bacterial groups based on previously described methods [10]. Changes in fecal bacteria community after fermentation are shown in Table 1. The numbers of bacterial groups across three treatments were analyzed using SAS/PROC GLM (SAS ver. 9.1; SAS Institute). Inclusion of inulin to fecal microbiota significantly increased the number of total bacteria, *Bacteroides* group, *Lactobacillus*, *Bifidobacteria*, and *Clostridium* strains when compared with the cellulose or control groups, whereas the cellulose group showed a significantly higher number of *Enterobacteria* and *Escherichia*

Table 1. Counts (log_{10} 16S rDNA gene copies/g of human feces) of different bacterial groups measured by quantitative real-time PCR.

Bacteria	Diet			SEM	*P*-value
	Control	Cellulose	Inulin		
Total bacteria	9.38 ± 0.23[b]	10.23 ± 0.04[a]	10.32 ± 0.06[a]	0.088	<0.01
Bacteroides group	7.10 ± 0.26[b]	8.59 ± 0.06[a]	8.87 ± 0.06[a]	0.100	<0.01
Lactobacillus	7.00 ± 0.14[c]	8.06 ± 0.03[b]	8.80 ± 0.07[a]	0.060	<0.01
Bifidobacteria	6.93 ± 0.39[b]	10.07 ± 0.06[a]	10.25 ± 0.05[a]	0.147	<0.01
Clostridium	7.74 ± 0.17[b]	7.84 ± 0.18[b]	8.58 ± 0.17[a]	0.101	<0.05
Enterobacteria	5.15 ± 0.24[b]	7.25 ± 0.05[a]	5.73 ± 0.14[b]	0.108	<0.01
Escherichia coli	4.74 ± 0.08[b]	5.95 ± 0.05[a]	4.43 ± 0.14[b]	0.062	<0.01
Lactobacillus : Enterobacteria	1.85 ± 0.11[b]	0.81 ± 0.05[c]	3.07 ± 0.08[a]	0.056	<0.01
Bifidobacteria : Enterobacteria	1.79 ± 0.30[c]	2.81 ± 0.06[b]	4.52 ± 0.09[a]	0.122	<0.01

Values are means ± SEM; $n = 5$. Means in a row with superscripts without a common letter differ.

coli strains when compared with the control or inulin group. Interestingly, a significantly greater ratio of *Lactobacillus* or *Bifidobacteria* to *Enterobacteria* was observed in fermentation with inulin. This ratio is regarded as an index of healthy human intestine, where a high index indicates greater resistance to intestinal disorders and improvement of soundness against opportunistic pathogens [3, 6]. This is in line with a previous *in vivo* study using a pig model, which showed an increased ratio of *Lactobacillus* to *Enterobacteria* induced by kiwifruit fiber [10]. Probiotics such as *Lactobacillus* and *Bifidobacteria* strains occur as natural intestinal flora and exhibit an ability to interfere with the virulence of different pathogens [7, 14]. Their beneficial effects include production of antimicrobial compounds and competition for pathogen-binding sites, resulting in well-balanced gut microbiota [8, 12].

Extracted bacterial DNAs were also applied to polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) using a Veriti 96-well thermal cycler (Applied Biosystems) with Takara Ex Taq polymerase (Takara, Japan) and the DCode system (Bio-Rad Lab., USA) with primers (forward, CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC and reverse, CGG TGT GTA CAA GAC CC), on polyacrylamide gels (8%) containing the urea–formamide gradient of 35–50% at a constant temperature of 60°C with modification of methods of Balan *et al.* [1]. Although there are some limitations of PCR-DGGE, including difficulty in separating bacterial species with similar DNA sequences, this method has been successfully used to study the taxonomy of gut microbiota in *in vivo* models [1, 10, 16].

The total number and intensity of DGGE bands were not significantly different across the three treatments (data not shown). However, the dendrogram of the similarity cluster analysis by BioNumerics ver. 7.1 (Applied Maths, Belgium) indicated the presence of three distinct clusters, which represented that each group has different bacterial microbiota (Fig. 1). Thirteen bands of specific interest on DGGE were extracted using the Wizard SV gel and PCR clean-up system (Promega, USA) and cloned into the pGEM-T easy

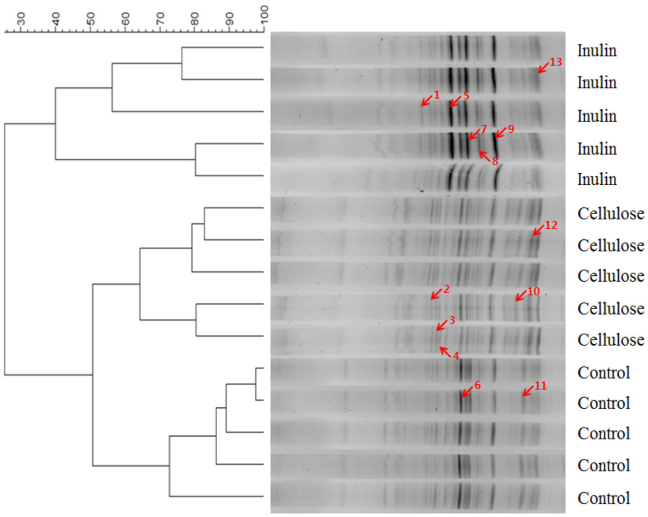

Fig. 1. Dendrogram of similarity cluster analysis for PCR-DGGE profiles of *in vitro* fermented human feces with no dietary fiber as a control and two dietary fibers (cellulose or inulin).

Bands were selected for identification of bacteria when they showed an obvious difference among the three groups. Arrows indicate the bands subjected to DNA sequencing.

Table 2. Identified bacterial species from DNA sequencing of the PCR-DGGE bands.

Band No.	Nearest neighbor	Query cover - similarity	Accession No.
1	*Akkermansia muciniphila* ATCC BAA-835	99 – 99	CP001071.1
2	Butyrate-producing bacterium PH07AY3	99 – 95	DQ144126.1
3	*Eubacterium eligens*	99 – 94	L34420.1
4	*Akkermansia muciniphila* ATCC BAA-835	99 – 100	NR_074436.1
5	Butyrate-producing bacterium SSC/1	99 – 96	AY305319.1
6	*Eubacterium rectale* ATCC 33656	99 – 99	CP001107.1
7	Butyrate-producing bacterium M104/1	99 – 99	AY305311.1
8	Butyrate-producing bacterium T1-815	99 – 99	AJ270476.2
9	Butyrate-producing bacterium P607 1	99 – 99	DQ144117.1
10	*Escherichia coli* O145:H28	99 – 99	CP006027.1
11	*Lachnospiraceae bacterium* 19gly4	99 – 99	AF550610.1
12	*Faecalibacterium prausnitzii* strain HTF-E	99 – 96	HQ457029.1
13	*Bifidobacterium* sp. HM5-5	99 - 95	AB649137.1

vector (Promega, USA) for DNA sequencing. The closest relatives of bands were obtained at GenBank with the BLAST program (Table 2). Nucleotide sequences of each band are available in the GenBank database under the accession numbers LC053681 to LC053693.

Interestingly, inclusion of inulin into fermentation increased the number and intensity of bands (No. 5, 7, 8, and 9) of butyrate-producing bacterial strains (Table 2). This tendency may explain the results of fatty acids determination by gas chromatography (Agilent Technologies 6890N GC system, USA) coupled with mass spectrometry (LECO Pegasus IV time-of-flight mass spectrometry, USA) using a capillary column (DB-5ms, 30 × 0.25 × 25). The relative butyrate production rates were significantly elevated in the inulin group (about 47.5% as percent of total fatty acids) when compared with the control (22.4%) or cellulose (7.4%) group. In addition, inulin tended to produce various kinds of fatty acids (C_4-C_{18}), resulting in detection of 17 kinds of fatty acids in the inulin group but only eight kinds of fatty acids in the other groups. It has been reported that fermentation of inulin-type fructans by colonic microbiota increases the formation of butyrate, which plays an important role in the metabolism of epithelial cells and maintenance of mucosal functions in the colon [19, 23]. Elevation of butyrate production may lead to enhanced gut health by creating a more acidic environment, which resists colonization of pathogens [21]. The reduction of *Enterobacteria* strains in the inulin group may be induced by end-products of inulin fermentation, such as butyrate, as well as antimicrobial compounds produced by enhanced beneficial bacteria. This is in accordance with the report of Castillo *et al.* [2], in which butyrate tended to promote a higher ratio of *Lactobacillus* to *Enterobacteria* strains in the gut. Moreover, the band of *Akkermansia muciniphila* strains on the DGGE gel of the inulin group is reported to regulate obesity and its associated metabolic disorders of mice [5].

The intestinal microbiota contains a complex community of microbes, which may be quantitatively and qualitatively diverse according to ethnic groups consuming different cultural diets. Until now, few studies have attempted to investigate the impact of prebiotic supplementation on *in vitro* fermentation using Korean fecal microbiota. Although further *in vivo* studies are needed to confirm the effect of dietary fiber, as *in vitro* fermentation is a batch system without absorption of metabolites and may not represent the complexity of human colonic microbiota, the system used in this study can be an effective and rapid method to evaluate the potential health benefits of dietary fiber in developing functional products. In addition, stool samples from patients with infectious conditions can be applied to this model to investigate how to improve host health.

Overall, this study has revealed that fermentation of inulin with Korean fecal microbiota results in prebiotic activity, by modulating bacterial communities and elevating the butyrate concentration, which is related to improvement of gut health.

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