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Effects of partially hydrolyzed guar gums of different molecular weights on a human intestinal *in vitro* fermentation model

Daisuke Sasaki,[1] Kengo Sasaki,[1,§] Aya Abe,[2] Makoto Ozeki,[2] and Akihiko Kondo[1,3,*]

Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan,[1] Nutrition Division, Taiyo Kagaku Co., Ltd., 1-3 Takaramachi, Yokkaichi, Mie 510-0844, Japan,[2] and RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan[3]

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Partially hydrolyzed guar gums (PHGGs) are prebiotic soluble dietary fibers. High molecular-weight PHGGs have rapid fermentation and high short-chain fatty acid (SCFA) productivity rates, compared to low molecular-weight PHGGs. Therefore, low molecular-weight PHGGs may have less pronounced prebiotic effects than high molecular-weight PHGGs. However, the effects of PHGGs of different molecular weights on the human intestinal microbiota, as well as their fermentation ability and prebiotic effects, have not been investigated. The aim of this study was to evaluate the effects of two PHGGs of different molecular weights, Sunfiber-R (SF-R; 20 kDa) and Sunfiber-V (SF-V; 5 kDa), on human colonic microbiota and SCFA production. A human intestinal *in vitro* fermentation model was operated by fecal samples with and without the PHGGs. The addition of 0.2% SF-R or SF-V increased the relative abundance of *Bacteroides* spp., especially that of *Bacteroides uniformis*. This increase corresponded to a significant ($p = 0.030$) and non-significant ($p = 0.073$) increase in propionate production in response to SF-R and SF-V addition, respectively. Both fibers increased the relative abundance of *Faecalibacterium* and stimulated an increase in the abundance of unclassified *Lachnospiraceae* and *Bifidobacterium*. In conclusion, the low molecular-weight PHGG exerted prebiotic effects on human colonic microbiota to increase SCFA production and bacteria that are beneficial to human health in a manner similar to that of the high molecular-weight forms of PHGG.

[**Key words:** Partially hydrolyzed guar gum; Intestinal *in vitro* fermentation model; Human colonic microbiota; Dietary fiber; Short-chain fatty acid]

The lumen of the human gastrointestinal tract contains trillions of bacteria that interact with each other and with the lumen. These interactions influence nutrient intake and metabolism (1). Dietary fibers influence the gut microbiota composition, exerting beneficial metabolic effects (2). Dietary fibers are polysaccharides with a minimum of 10 monosaccharide units or oligosaccharides containing 3–9 monosaccharide units (3). Human digestive enzymes do not hydrolyze dietary fibers, whereas gut microbes can process them and produce metabolites such as short-chain fatty acids (SCFAs).

Guar gum is a natural polymer extracted from the seeds of *Cyamopsis tetragonolobus* (4). Partially hydrolyzed guar gums (PHGGs) are soluble dietary fibers. They have a linear chain of β-1,4-linked D-mannose and α-D-galactose residues that are 1,6-linked to alternating mannose residues (5). In patients with irritable bowel syndrome, PHGG intake improves stool form and alleviates abdominal pain (6). However, *in vivo* and *in vitro* studies on the effects of PHGGs on human intestinal bacteria have shown varying results. In one study, PHGG increased the growth of gut microbiota such as *Bacteroides ovatus*, *Clostridium coccoides*, and *Clostridium butyricum in vitro* and did not affect the growth of *Bifidobacterium* (7). Another study found that PHGG supported the growth of *Bacteroides* and *Parabacteroides* in a human intestinal *in vitro* fermentation model (8). Multiple *in vivo* studies have found that PHGG consumption increases the abundance of *Bifidobacterium*, *Ruminococcus*, and *Megasphaera* and reduces that of the *Bacteroides* members (9,10). In healthy human participants, PHGG intake has been associated with an increase in the abundance of *Fusicatenibacter*, *Bacteroides*, *Ruminococcus*, and *Faecalibacterium* and a reduction in the abundance of *Blautia*, *Roseburia*, and *Lachnospiraceae*, along with an increase in the concentration of acetate and butyrate (5).

The PHGGs have already been marketed as prebiotic food ingredients in various forms. The molecular weights of PHGGs substantially affect their physical properties. When used as supplements, high molecular-weight PHGGs are difficult to dissolve in water and ingest and are difficult to incorporate into foods such as bread and noodles. Conversely, low molecular-weight PHGGs are more readily incorporated into foods. In a previous study, PHGGs of different molecular weights presented different SCFA productivity and fermentation rates in *in vitro* fecal cultures (11). Briefly, culture media containing PHGGs of different molecular weights, 15, 20, 400, and 1100 kDa, were inoculated into human feces and incubated for 24 h; 400 kDa PHGG presented the fastest fermentation rate and highest SCFA productivity. Therefore, low molecular-weight PHGGs may have less pronounced prebiotic effects than high molecular-weight PHGGs. However, there is a lack of studies

* Corresponding author: Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan.
E-mail address: akondo@kobe-u.ac.jp (A. Kondo).
§ Present address. BioPalette Co., Ltd., 6-3-7 Minatojima Minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan.

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on the effects of PHGGs of different molecular weights on the human intestinal microbiota and the fermentation ability and prebiotic effects of PHGGs with high applicability in food processing.

The Kobe University Human Intestinal Microbiota Model (KUHIMM) targets colonic microbiota by utilizing a batch fermentor, which is versatile and accessible (12,13). The batch fermentation system has limitations in terms of microbiological control, such as limited microbial growth and reconstruction of microbial structure (14). To overcome these limitations and enable the growth of most bacterial species present in the original fecal inoculum, the KUHIMM uses a Gifu anaerobic medium (GAM), which supports the growth of various gut microorganisms (15). In addition, the KUHIMM has a broad pH range of 5.0–7.5, similar to that of the human colon, which fluctuates primarily between 5.0 and 8.0 (16). Therefore, the KUHIMM maintains bacterial abundance and diversity at levels comparable to those in the original fecal inoculum (17). At a stable neutral pH of 6.5, the KUHIMM helps identify the relationship between the types of glucosidic linkage in dietary fibers and their fermentation ability by the human colonic microorganisms (18). In the present study, two PHGGs were used: Sunfiber-R (SF-R), with a high molecular-weight of 20 kDa, and Sunfiber-V (SF-V), with a low molecular-weight of 5 kDa. To evaluate the discrepancies between previous *in vivo* and *in vitro* study results, in this study, we investigated the effects of the two PHGGs on the structure of the intestinal microbiota and on SCFA production using the KUHIMM, which employs fecal inoculum from human donors.

MATERIALS AND METHODS

Preparation of PHGGs Commercial PHGGs (SF-R and SF-V; Taiyo Kagaku Co., Ltd., Tokyo, Japan) were used in this study (Supplementary Table S1). Their molecular weights were measured using high-performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan) according to a previously described procedure (19,20). For the HPLC analysis, we used TSKgel G4000PWXL and G2500PWXL columns (150 mm × 6.0 mm inner diameter; Tosoh, Tokyo, Japan) and an RID-20A detector (Shimadzu Corporation). The operation parameters were as follows: Milli-Q water at 0.3 mL/min as the eluent, column temperature of 80 °C, and injection volume of 20 μL. The liquid sample was diluted in Milli-Q water at 1% (w/w). The P-82 Shodex Standard Kit (Showa Denko, Tokyo, Japan) was used as the standard to generate the calibration curve.

Collection of fecal samples from healthy humans Fecal samples were collected from 15 healthy human volunteers who had not been treated with antibiotics for at least 6 months prior to sample collection. The inclusion criteria were as follows: Japanese ancestry, no pre-existing illnesses (according to patient interviews), aged 20–60 years, and non-smoker. The study design was approved by the institutional ethics review board of Kobe University Hospital, Clinical and Translational Research Center, and all participants provided written informed consent before fecal specimen collection. Each fecal sample was collected and maintained in an anaerobic condition with a BD BBL Culture Swab (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The Culture Swab was stored in room temperature and used within 48 h. This study was conducted following the principles of the Declaration of Helsinki.

Operation of the KUHIMM The KUHIMM was used with or without prebiotic addition, using a Bio Jr.8 fermentor (ABLE, Tokyo, Japan), as previously described (17). The medium (100 mL) was based on GAM (Nissui Pharmaceutical Co., Tokyo, Japan). The culture media in the fermentors were always filled and bubbled with an anaerobic gas mixture (N_2:CO_2 = 80:20). The cultures were started by inoculating individual fecal suspensions (100 μL) in each fermentor. Fecal suspensions were prepared as 0.5 g feces in 2.0 mL PBS buffer (Nacalai Tesque, Kyoto, Japan). The incubation temperature was 37 °C. The culture broth was stirred at 300 rpm and continuously purged with the gas mixture to maintain anaerobic conditions. To analyze the fermentation rate and ratio of PHGGs, 100 mL of the medium was buffered to a pH of 6.5 using phosphate buffer (0.1 M NaH_2PO_4:0.1 M Na_2HPO_4 = 2:1). SF-R and SF-V were added to each fermentor at 1.0% (10 g/L) at 18 h after culture initiation. From the findings of previous KUHIMM experiments, the number of cultured colonic microbiota plateaus after approximately 18 h of incubation (18). Hence, PHGG was added at this time point. The culture broth from the KUHIMMs (n = 3) was sampled at 42 h after culture initiation and analyzed. Based on the analysis results, SF-R and SF-V were added to each fermentor at 0.2% (2.0 g/L) to investigate the effects of PHGGs on colonic microbiota. The culture broth from the KUHIMMs (n = 15) with or without PHGGs was sampled from the fermentors 48 h after culture initiation. Fecal and culture samples were stored in a freezer (−20 °C) until use.

Measurement of dietary fiber concentrations To calculate the fermentation rate and ratio, the concentration of indigestible dietary fibers in the culture media was measured through enzymatic digestion, using the TDF-100A Total Dietary Fiber Assay Kit (Sigma–Aldrich Co., St. Louis, MO, USA), as described previously (18). The residual indigestible content after enzymatic digestion was measured using HPLC equipped with two TSKgel G2500PWxl columns (Tosoh).

DNA extraction Genomic DNA was extracted from the suspension of fecal inoculum and culture samples of the KUHIMMs at 48 h, as described previously (21). The DNA was eluted into TE buffer (10 mM Tris–HCl and 1.0 mM ethylenediaminetetraacetic acid) and stored in a freezer (−20 °C) until use.

16S sequencing The V3–V4 region of bacterial 16S rRNA genes was amplified using the extracted DNA samples as the template, as previously described (22,23). Following the manufacturer's instructions, PCR was performed with an Nextera XT index adapter added to the gene sequence (Illumina Inc., San Diego, CA, USA). Amplicons were purified using AMPure XP DNA purification beads in accordance with the manufacturer's instructions (Beckman Coulter, Brea, CA, USA). The concentration of the purified amplicons was measured using a Qubit fluorometer (Thermo Fisher Inc., Waltham, MA, USA). The amplicons were pooled at an equimolar concentration of 5 nM. The 16S rRNA genes and internal PhiX control (Illumina) were analyzed for paired-end sequencing using MiSeq (Illumina) with Reagent Kit v3 (Illumina) for 600 cycles. Pair-end reads with a Q score of 20 or higher were combined using automated CASAVA 1.8 pair-end demultiplexing FASTQ, with the FASTQ Generation in Basespace Sequence Hub. The sequences were subjected to quality control and corrected with the DADA2 pipeline using QIIME 2 version 2022.2 (24). The operational taxonomic units (OTUs) were classified using the naive Bayes classifier trained on the Greengenes 13_8 99% OTU full-length sequence database. The OTUs and taxonomic metadata were used for α-diversity estimation.

Quantification of 16S rRNA genes Real-time PCR was conducted as previously described (21). Using a LightCycler 96 system (Roche, Basel, Switzerland), the total bacterial number was used to quantify the total bacterial 16S rRNA genes using the primer sets targeting all eubacteria (25,26).

Measurement of SCFAs The concentrations of acetate, propionate, butyrate, lactate, and succinate were determined as previously described (21). An HPLC system (Shimadzu Corporation) equipped with a RID-10A refractive-index detector (Shimadzu Corporation) and an Aminex HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used to detect the SCFAs. The HPLC system was operated at a flow rate of 0.6 mL/min at 65 °C; 5 mM H_2SO_4 was used as the mobile phase.

Statistical analyses All statistical analyses in this study were performed using Prism 8 (GraphPad Software, Inc., San Diego, CA, USA). Results with $p < 0.05$ were considered statistically significant.

Data availability All 16S rRNA gene sequences obtained in this study have been deposited at the MG-RAST server (27) as "Model Culture System of Human Colonic Microbiota_PHGGs" under accession numbers mgm4927749.3–mgm4927808.3 (MG-RAST, https://www.mg-rast.org/).

RESULTS

Fermentation rate and fermentation ratio of PHGGs The fermentation rate of the two PHGGs was evaluated using the KUHIMM and compared with that of other dietary fibers obtained in our previous study (18). At 24 h after adding 1.0% PHGGs (after 42 h of fermentation), the fermentation ratio (%) was determined. The fermentation rate (g/L/h) was also determined within 24 h. According to this analysis, SF-R exhibited higher fermentation and degradation rates than SF-V; however, the difference in the effects on SF-V and SF-R was not significant. The fermentation rate and fermentation ratio for PHGGs were relatively higher than those for other dietary fibers such as indigestible dextrin and isomaltodextrin (Table 1).

Effects of PHGGs on the human intestinal microbiota structure in the KUHIMM The effect of the PHGGs on human colonic microbiota was evaluated. The PHGGs (0.2%) were administered to the fecal sample inoculum, which was then fermented for 48 h to construct the KUHIMM; KUHIMM without PHGG administration was used as the control (CUL). At 48 h, the number of eubacteria was determined using real-time PCR. The eubacterial copy number was $3.44 \pm 1.84 \times 10^{11}$ copies/mL in CUL; however, the eubacterial copy number did not significantly change with

TABLE 1. Fermentation ratio and fermentation rate of the dietary fibers.

Dietary fiber	Fermentation rate (g/L/h)	Fermentation ratio (%)	Reference
SF-R	0.40 ± 0.15	94.1 ± 7.15	This study
SF-V	0.32 ± 0.11	85.5 ± 19.8	This study
Indigestible dextrin	0.21 ± 0.09	32.8 ± 13.8	18
Isomaltodextrin	0.30 ± 0.15	57.1 ± 23.5	18

SF-R, Sunfiber-R; SF-V, Sunfiber-V. In the KUHIMM experiment of Reference 18, fecal samples from the healthy volunteers enrolled in the current study, collected at a different date, were used.

the addition of PHGGs ($p > 0.05$; Mann–Whitney *U*-test, Supplementary Fig. S1).

After 48 h of fermentation, bacterial 16S sequencing of the 15 fecal inoculum samples and the corresponding KUHIMM samples with or without PHGGs yielded 2,527,402 quality reads (Supplementary Table S2). The OTU count and Chao1 value for species richness were lower in the CUL than in the original fecal sample groups (FEC; $p = 0.002$ and 0.002, for OTUs and Chao1, respectively; Mann–Whitney *U*-test). However, there was no significant difference in the OTU count between the CUL and the SF-R or SF-V group ($p = 0.519$ and 0.300, respectively; Mann–Whitney *U*-test). The Shannon index for species diversity was lower in the CUL group than in the FEC group ($p = 0.0002$; Mann–Whitney *U*-test). However, there was no significant difference in the values between the CUL and SF-R or SF-V group ($p = 0.935$ or 0.595, respectively; Mann–Whitney *U*-test). The Simpson index for species diversity was lower in the CUL group than in the FEC group ($p = 0.003$; Mann–Whitney *U*-test); however, no significant difference was found in the values between the CUL and SF-R or SF-V group ($p = 0.870$ and 0.903, respectively; Mann–Whitney *U*-test). These findings confirmed that the diversity of the intestinal microbiota did not change upon the addition of 0.2% PHGG in the KUHIMM. On the other hand, considerable changes in bacterial composition were detected.

The relative abundance of *Bacteroides* members increased in the KUHIMM with the addition of SF-R or SF-V, compared with that in the KUHIMM not supplemented with PHGGs ($p = 0.048$ and 0.048 for SF-R and SF-V, respectively; Wilcoxon signed-rank test; Figs. 1 and 2A). Among the members of *Bacteroides*, the abundance of *Bacteroides uniformis*-related organisms tended to increase with the addition of PHGGs compared with that in the CUL group ($p = 0.394$ and 0.009, respectively; Wilcoxon signed-rank test; Fig. 2B and C). The low molecular-weight PHGG markedly increased the abundance of *B. uniformis* (Fig. 2C). The abundance of other *Bacteroides*-related organisms, such as *B. plebeius*, *B. ovatus*, *B. fragilis*, *B. eggerthii*, *B. caccae*, and *B. acidifaciens*, did not increase with the addition of PHGGs ($p > 0.05$; Wilcoxon signed-rank test). In addition, the relative abundance of bacteria related to *Faecalibacterium* increased in the KUHIMM with the addition of SF-R or SF-V, compared with that in the CUL group ($p = 0.042$ and 0.049, respectively; Wilcoxon signed-rank test; Fig. 3). The addition of SF-R increased the relative abundance of unclassified *Lachnospiraceae* ($p = 0.013$; Wilcoxon signed-rank test); however, this effect on unclassified *Lachnospiraceae* was not significant when SF-V was added ($p = 0.208$; Wilcoxon signed-rank test). The addition of SF-V increased the relative abundance of bacteria related to *Bifidobacterium* ($p = 0.010$; Wilcoxon signed-rank test); however, this effect on *Bifidobacterium* was not significant when SF-R was added

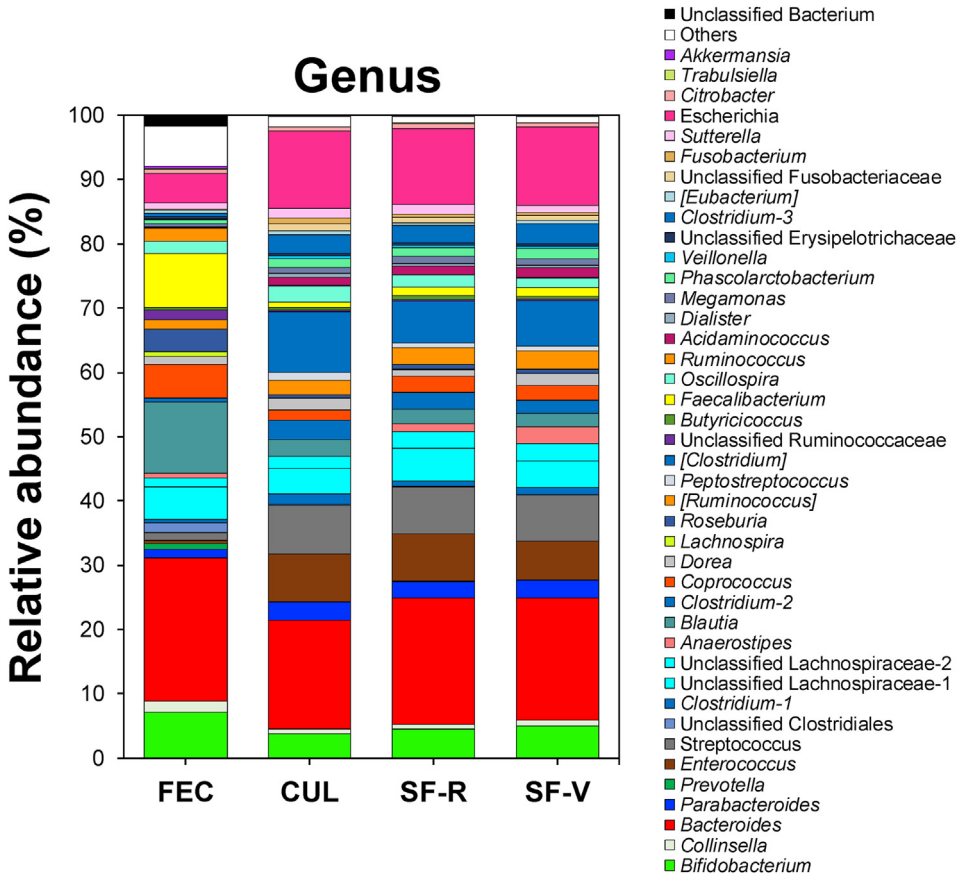

FIG. 1. Effect of 0.2% partially hydrolyzed guar gum (PHGG) on the intestinal microbiota composition. Genus-level classifications of bacteria from original fecal samples (FEC) and the human intestinal *in vitro* fermentation model (KUHIMM) without PHGG (CUL) and with PHGGs, including Sunfiber-R (SF-R) and Sunfiber-V (SF-V), after 48 h of fermentation. Average relative abundances are shown for 15 samples. Genera with a low abundance (<1.0%) and low similarity (<99%) were included in the others and unclassified bacteria groups, respectively.

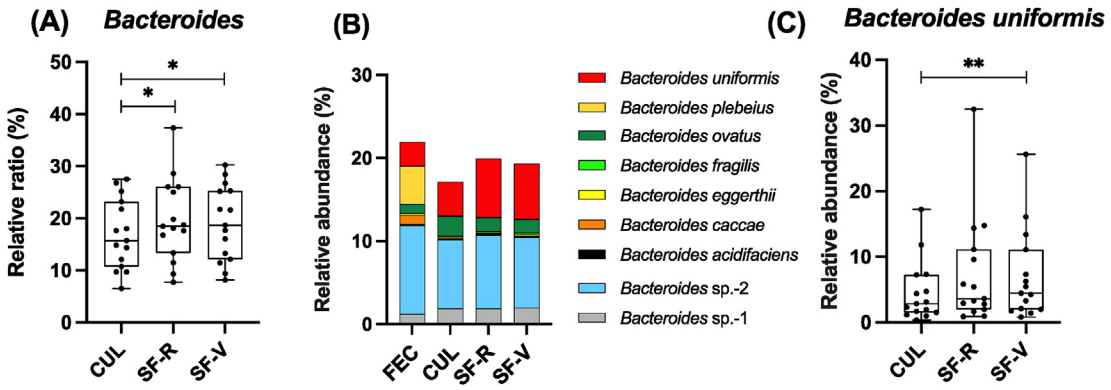

FIG. 2. Relative abundance of the genus *Bacteroides*. (A) Genus *Bacteroides*, (B) *Bacteroides* species, and (C) *Bacteroides uniformis* in the KUHIMM samples without PHGG (designated as CUL), with 0.2% Sunfiber-R (SF-R) or Sunfiber-V (SF-V), and in original fecal samples (FEC). (A, C) Box and whisker plot. $*p < 0.05$, $**p < 0.01$; Wilcoxon signed-rank test. (B) The average value of 15 samples.

($p = 0.229$; Wilcoxon signed-rank test). For the other genera detected in Fig. 1, no statistical changes were detected in the 15 fecal inoculum samples and the corresponding KUHIMM samples with or without PHGGs.

Effect of the PHGGs on SCFA production in the KUHIMM SCFAs are major end-products of gut microbiota metabolism and exert a wide range of effects on host physiology (28). The production of SCFAs was evaluated in the CUL group and in the KUHIMM, with the addition of 0.2% SF-R or SF-V after 48 h of fermentation (Fig. 4). The addition of SF-R increased the production of propionate compared with that in the CUL group ($p = 0.030$; Wilcoxon signed-rank test); however, propionate production did not significantly increase with the addition of SF-V, although the production tended to increase ($p = 0.073$; Wilcoxon signed-rank test; Fig. 4B). The production of acetate and butyrate remained unchanged with the addition of SF-R or SF-V ($p > 0.05$; Wilcoxon signed-rank test; Fig. 4A, C).

DISCUSSION

In a previous study, PHGGs of four molecular weights (15, 20, 400, and 1100 kDa) were fermented using a batch *in vitro* fermentation system, but the fermentability did not vary significantly among the fractions (11). Herein, two PHGGs of high and low molecular weights (SF-R 20 kDa and SF-V 5 kDa, respectively) showed relatively high fermentation rates in the human intestinal *in vitro* fermentation model, KUHIMM. The PHGGs are structurally composed of β-1,4-linked mannose units with α-1,6-linked galactose units as side chains (5). Dietary fibers with a high content of 1,4- and 1,6-glycosidic linkages are easily hydrolyzed by human colonic microbiota. In addition, other indigestible dietary fibers containing 1,2- and 1,3-glycosidic linkages are known to be resistant to degradation by colonic microbiota (18). Therefore, the two PHGGs with a high content of 1,4- and 1,6-glycosidic linkages and no content of 1,2- and 1,3-glycosidic linkages have high fermentability regardless of the difference in the molecular weight (29).

Both PHGGs stimulated an increase in the abundance of the beneficial *Bacteroides* members, particularly *B. uniformis*, consistent with the results of previous studies (7,8). Some strains of *B. uniformis* reduce the viscosity of guar gum, possibly via a reduction in the polysaccharide chain length (30). The low molecular-weight PHGG SF-V extensively increased *B. uniformis* abundance, suggesting that PHGG with a low molecular weight and low viscosity can promote the growth of *B. uniformis*. Oral intake of *B. uniformis* CECT 7771 reportedly alleviated weight gain and improved lipid metabolism in obese mice (31). The administration of PHGGs can prevent obesity by altering the colonic microbiota, with the aid of voluntary exercise (32). In addition, the administration of the oligosaccharide α-cyclodextrin reportedly increased the abundance *Bacteroides* members, particularly *B. uniformis*, and production of SCFAs, such as propionate and acetate; an increase in the abundance of *B. uniformis* improves exercise performance in humans and mice (33).

SCFAs have attracted attention with developments of prebiotics and probiotics aimed at improving colonic and systemic human

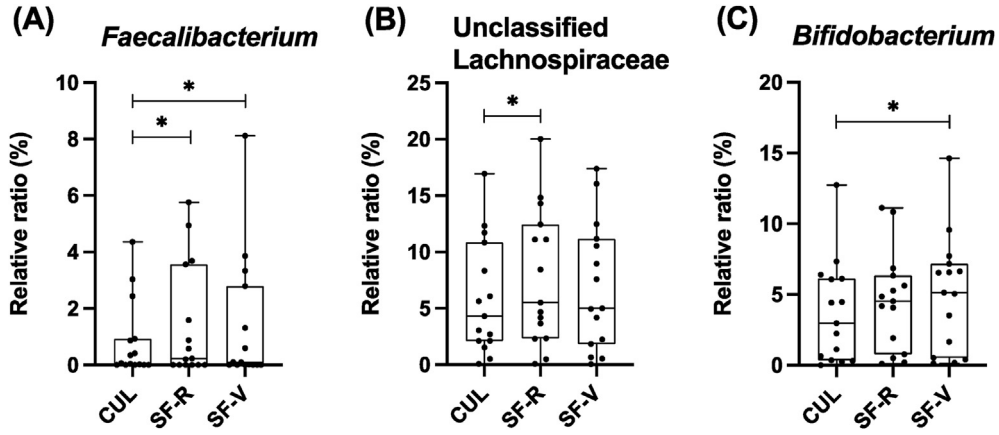

FIG. 3. Box and whisker plot of the various microbial species in the KUHIMM samples. (A) Genus *Faecalibacterium*, (B) unclassified *Lachnospiraceae*, and (C) genus *Bifidobacterium* in the KUHIMM samples without PHGG (designated as CUL) and with 0.2% Sunfiber-R (SF-R) or Sunfiber-V (SF-V). $*p < 0.05$; Wilcoxon signed-rank test.

health, and propionate, which is primarily produced by the fermentation of dietary fibers, has been reported to have various health benefits (34). Propionate is formed by the action of the gut bacteria via two pathways, the succinate and propanediol pathways (35). The succinate pathway is often found in members of the phylum Bacteroidetes, including *Bacteroides* (35). The relative abundance of Bacteroidetes members correlates with the production of propionate *in vitro* (18). The increase in propionate production was considerably high following the addition of both types of PHGGs and the increase in the abundance of *Bacteroides* members. However, the effect of SF-V on propionate production was not significant. The difference between the high and low molecular-weight PHGGs influenced propionate production by the gut microbiota and *Bacteroides* members. These results suggest that the total amount of PHGG degradation products and the total abundance of *Bacteroides* affect the amount of propionate produced.

Following PHGG addition, an increase in the abundance of *Faecalibacterium*, a dominant acetate-utilizing butyrate producer, was observed, consistent with the results of a previous interventional study in healthy human participants (5). *Faecalibacterium* does not directly utilize PHGGs. *Faecalibacterium* degrades various β-mannooligosaccharides, which are derived from the primary mannanolytic activity of other colonic microorganisms (36). A metabolically complementary relationship exists between *Bacteroides thetaiotaomicron*, an acetate producer, and *Faecalibacterium prausnitzii*, an acetate consumer and butyrate producer (37). Therefore, the addition of PHGGs indirectly influences the abundance of *Faecalibacterium*. On the other hand, the genera *Anaerostipes* and *Eubacterium*, known as lactate-utilizing butyrate producers, did not significantly increase upon the addition of PHGGs.

Here, the addition of SF-R (high molecular-weight = 20 kDa) and SF-V (low molecular-weight = 5 kDa) increased the abundance of unclassified *Lachnospiraceae* and *Bifidobacterium*, respectively. The *in vitro* increase in the abundance of *Bifidobacterium* with PHGG addition corresponded to the *in vivo* results of studies in healthy human volunteers (9,38). Nevertheless, a reduction in *Lachnospiraceae* abundance was observed in an *in vivo* study on human volunteers (9). These variations could be attributed to the differences in the molecular weights of the PHGGs. Not all *Bifidobacterium* strains ferment PHGGs *in vitro* (7); some utilize the products derived from PHGG degradation by other bacteria, such as *Bacteroides*. Bacterial cross-feeding occurs in the human gut, and there are trophic interactions between bacteria from *Lachnospiraceae* and the bifidobacterial groups (39). In addition, there are syntrophic interactions between *Bacteroides* and *Bifidobacterium* species in the metabolism of various glycans (40). However, strains such as

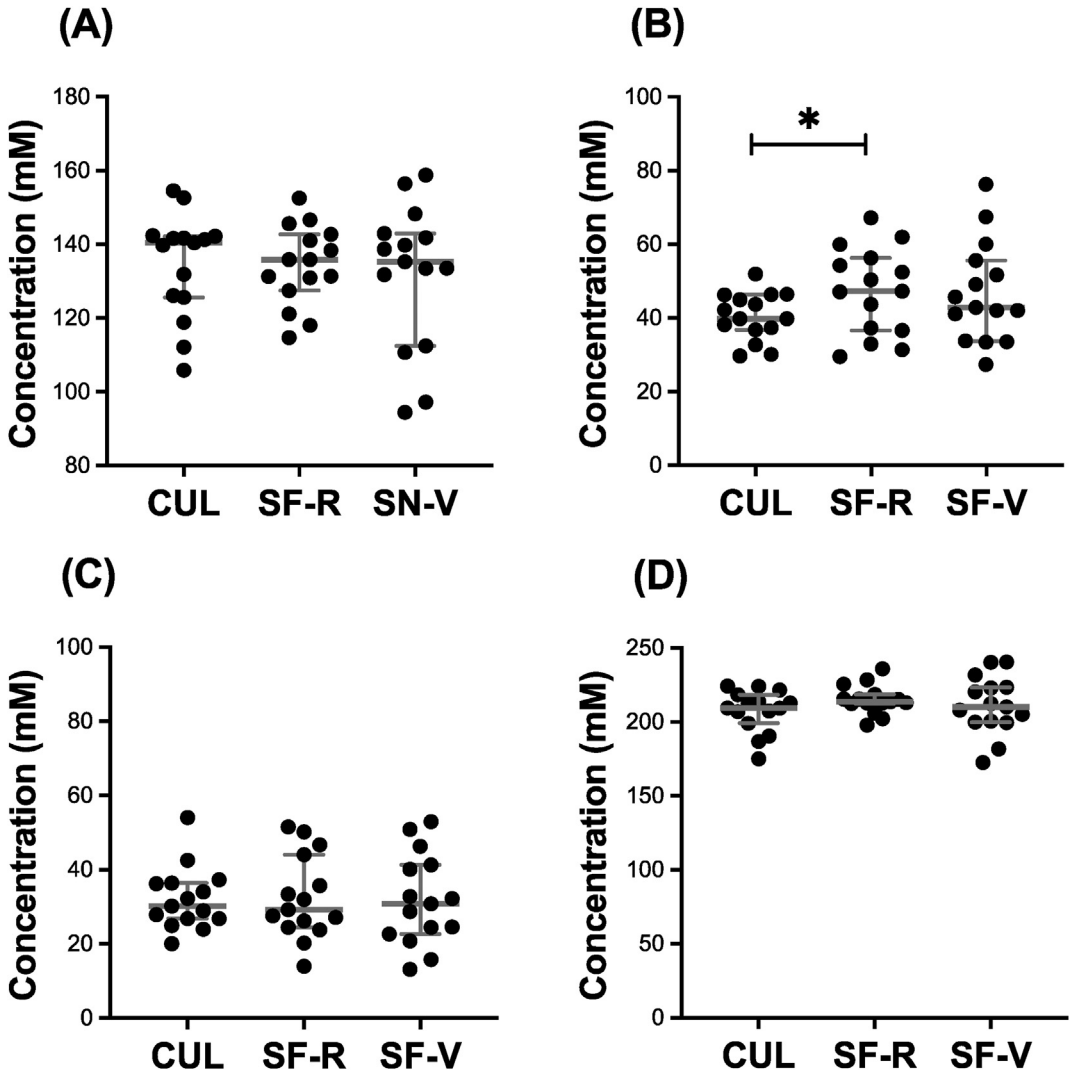

FIG. 4. Concentration of various metabolites in fecal samples. Concentrations of (A) acetate, (B) propionate, (C) butyrate, and (D) total short-chain fatty acids (SCFAs) in the KUHIMM culture supplemented with or without 0.2% PHGG, i.e., SF-R or SF-V, after 48 h of fermentation. Asterisk indicates significant differences (*$p < 0.05$) from the control (KUHIMM samples without PHGG -CUL) values as determined using Wilcoxon signed-rank test. Data are illustrated as median and interquartile range (25th–75th percentile).

Bifidobacterium adolescentis ATCC 15703 harbor mannanase, which degrades guar gum galactomannan (41). Therefore, it is possible that some *Bifidobacterium* strains directly utilize PHGG. The differences in the molecular weights of PHGGs could influence the affinity of the PHGGs to some bacteria, altering the intestinal microbiota composition and their metabolite profiles.

In another study, the molecular weight of PHGGs was positively correlated with acetate production and negatively correlated with propionate production (11). PHGG of 400 kDa exhibited the fastest fermentation rate and highest SCFA productivity; however, there was no significant difference in the fermentability of 20 kDa and 1100 kDa PHGGs. The aforementioned study included only 3 fecal samples from human subjects, and the effect of PHGGs on colonic microbiota was not analyzed. In contrast, the current study demonstrated that with the individual colonic microbiota constructed in an *in vitro* culture system with high reproducibility, both 5 kDa SF-R and 20 kDa SF-V increased the prevalence of beneficial bacteria such as *Bacteroides*, *Faecalibacterium*, and *Bifidobacterium*, promoting SCFA production and indicating that both exert prebiotic effects. The present study shows that even a low molecular-weight PHGG (SF-V), which is more easily incorporated into food, maintains the high fermentability, changes the colonic microbiota, and promotes metabolite production comparable to those of high molecular-weight PHGGs.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2023.04.002.

Ethics approval All participants provided written informed consent before specimen collection. The study was approved and performed in accordance with the guidelines of the institutional ethics review board of Kobe University Hospital, Clinical and Translational Research Center. This study was performed in compliance with the principles of the Declaration of Helsinki.

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KS, DS, and AA conceived and designed the study. DS and KS conducted the experiments and analyzed the data. DS, KS, AA, MO, and AK wrote the manuscript. All authors read and approved the manuscript.

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