Dietary intake of yacon roots (*Smallanthus sonchifolius*) affects gut microbiota and fecal mucin and prevents intestinal inflammation in mice

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Consumption of yacon (*Smallanthus sonchifolius*) is associated with beneficial effects such as prevention of metabolic diseases. Yacon root is known to contain various bioactive components including indigestible carbohydrates, but the alteration of intestinal environment after treatment with yacon has not been fully investigated. This study investigated yacon-containing diet effects on the intestinal environment in mice, including microbial composition, short-chain fatty acid levels, and mucus content. After mice were administered yacon-containing diet for 4 weeks, 16S rRNA gene sequencing analyses revealed their fecal microbiota profiles. Organic acid concentrations in cecal contents were measured using an HPLC system. Compared to the control group, yacon-containing diet-received mice had significantly higher the concentrations of succinic acid, lactic acid, acetic acid, and propionic acid. The fecal mucin content was also higher in yacon-containing diet-received mice. Results of 16S rRNA gene sequencing analyses showed that the relative abundances of 27 taxa differed significantly in yacon-containing diet-received mice. Furthermore, results show effects of yacon administration on intestinal inflammation using 2,4,6-trinitrobenzene sulfonic acid induced colitis model in mice. Increased colonic damage and myeloperoxidase activity after 2,4,6-trinitrobenzene sulfonic acid treatment were suppressed in yacon-containing diet-received mice. Results suggest that oral intake of yacon root modulates the intestinal environment, thereby inhibiting intestinal inflammation.

Key Words: 16S rRNA, colitis, fructooligosaccharide, goblet cell, tumor necrosis factor-α

Yacon (*Smallanthus sonchifolius*), a native plant of the Andean highlands cultivated for its tuberous roots, contains various bioactive components such as dietary fiber and polyphenolic compounds.[1] In contrast to most edible starch-storing roots, yacon stores its carbohydrates in the form of β-(2→1) fructooligosaccharides (FOS). In fact, FOS are carbohydrates found naturally in plants of many types, but never in concentrations as high as those found for yacon roots.[2] The roots are consumed as dietetic food and are used as traditional medicine to prevent and treat metabolic diseases including hypertension and diabetes mellitus.[3,4]

Dietary fiber, which comprises complex polysaccharides resistant to digestion by amylases and glycoamylases in the small intestine, is divisible into two groups: insoluble and soluble. Of these, insoluble dietary fiber promotes colonic motility and gastrointestinal function mainly through physical bulking effects. By contrast, soluble dietary fiber including FOS has shown diverse health benefits both locally in the gastrointestinal tract and systemically throughout the body through modulation of gut microbial composition.[5–7] Although the FOS content of yacon is particularly high, the alteration of colonic microbiota after treatment with yacon has not been fully investigated.

A major function of gut microbiota is the fermentation of indigestible compounds such as dietary fiber, which leads to production of short-chain fatty acids (SCFAs). Accumulated evidence indicates that SCFAs have the most important physiological effects on the colonic mucosa, such as mucus secretion and increased motility.[8] Moreover, SCFAs have been shown to ameliorate various diseases including colorectal cancer and inflammatory bowel disease (IBD).[9–11] Actually, IBD, chronically relapsing disorders of the gastrointestinal tract, includes ulcerative colitis and Crohn's disease. Although the etiology of IBD remains poorly understood, cumulative data of clinical and experimental study suggest that dietary factors play important roles in the pathogenesis and clinical course of IBD. Higher consumption of fat is associated with increased risk for IBD, whereas high fiber, fruit, and vegetable intake are associated with decreased IBD risk.[12,13]

Given this context, we investigated the precise effects of yacon intake on gut microbiota using 16S rRNA gene sequencing analyses. Additionally, we analyzed the effects of yacon intake on 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, which shares both immunological and pathological features with human IBD.

Materials and Methods

Yacon preparation. Yacon was provided by Japan Agricultural Cooperatives of Nonoichi (Ishikawa, Japan). The yacon roots were peeled, sliced, and lyophilized. The lyophilized yacon roots were then ground to a fine powder using a cooking mixer and were sieved through a 300-μm mesh. The nutritional composition of the yacon sample, excluding dietary fiber, was analyzed by Ishikawa Health Service Association (Ishikawa, Japan). Dietary fiber contents were measured using a Dietary Fiber Assay kit (Fujifilm Wako Pure Chemical Corp., Osaka, Japan)

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Table 1. Nutritional composition of yacon sample

Constituent	Content
Protein (%)	2.9
Fat (%)	0.8
Carbohydrate (%)	79.8
Dietary fiber (%)	9.0
Ash (%)	3.1
Moisture (%)	4.4
Energy (kcal/100 g)	373.0

Energy was calculated based on Atwater coefficients (protein 4 kcal/g, fat 9 kcal/g, carbohydrate 4 kcal/g).

according to the manufacturer's instructions. The nutritional composition of the yacon sample is shown in Table 1.

Animals. Five-week-old male C57BL/6J mice used for this study were obtained from Sankyo Labo Service Corp. (Tokyo, Japan). The mice were kept at 18–24°C and 40–70% relative humidity, with a 12-h light/dark cycle. They were allowed free access to water and diet (AIN-76A; Research Diets, Inc., New Brunswick, NJ) for one week during their acclimatization period. Experiment procedures were conducted in accordance with the NIH guidelines for animal experimentation. All experiment protocols were approved by the Animal Care Committee of Ishikawa Prefectural University (permission number 30-14-9).

After acclimatization, the mice were fed one of the following diets: an AIN-76A-based control diet (Control diet, CD), an AIN-76A supplemented with 10% (w/w) yacon sample (Yacon-containing diet, YD) for 30 days. The CD approached the macronutrient content of YD by adding the following nutrients: 0.29% milk casein, 0.08% corn oil, 7.98% corn starch, and 0.9% cellulose. After 28 days of feeding, fresh fecal samples were obtained from each mouse for microbiota analyses. Thereafter, the mice were killed under anesthesia. Their cecal contents and colon were collected immediately.

Induction of colitis. For induction of TNBS-induced colitis, 100 mg/kg TNBS (Sigma-Aldrich Corp., St. Louis, MO) dissolved in 30% ethanol was administered intrarectally to anesthetized (ketamine/xylazine) mice via a catheter.[14] The sham group received 30% ethanol. Two days after TNBS administration, the mice were killed humanely. Then the colon damage was graded in terms of the presence of visible damage, serosal adhesions, diarrhea, strictures, and bowel wall thickening, as described in an earlier report.[15] All scoring was performed by the same individual under blind conditions to prevent observer bias.

Histological evaluation. Specimens of the distal colon were fixed in 10% neutral buffered formalin or in Carnoy's solution for 4 h. After fixation, specimens were embedded in paraffin, cut to 3-μm thickness using a microtome, and mounted on MAS-coated slides. Formalin-fixed and Carnoy-fixed sections were stained respectively with hematoxylin/eosin (HE) and alcian blue/periodic acid Schiff (AB/PAS).

Determination of organic acid concentrations in cecal contents. The organic acid concentrations in cecal contents were measured using a high performance liquid chromatography (HPLC) system according to the method described by Ushida *et al.*[16] In brief, a portion of the cecal contents (20–30 mg) was suspended with 0.5 ml of 14% perchloric acid to eliminate protein. After centrifugation at 10,000 rpm at 4°C for 5 min, the supernatant was filtered through a cellulose acetate membrane filter with 0.45 μm pore size. Then an aliquot of the resultant sample solution was injected into the HPLC system for analysis.

Determination of fecal mucin. Feces from the 48-h collection were lyophilized and powdered (Multi-beads Shocker machine; Yasui Kikai Corp., Osaka, Japan). Fecal mucin contents

were ascertained using a fluorometric assay kit (Fecal Mucin assay kit; Cosmo Bio Co., Ltd., Tokyo, Japan). Assays were performed according to the manufacturer's instructions. Fluorescence was detected with excitation at 336 nm and emission at 383 nm using a plate reader (Infinite M200; Tecan Group Ltd., Mannedorf, Switzerland).

Microbiota analysis by 16s rRNA sequencing. Fecal samples were collected, placed into tubes, and kept at −80°C until further use. Bacterial genomic DNA was extracted from the fecal samples using a kit (QuickGene DNA Tissue kit SII; Kurabo Industries Ltd., Osaka, Japan) with a nucleic acid extraction machine (QuickGene-Mini80; Kurabo Industries Ltd.). Preparation of the library for DNA sequencing using a desktop sequencer (MiSeq; Illumina Inc., San Diego, CA) was conducted according to the protocol with minor modifications, as described in an earlier report.[17] Briefly, the V3–V4 region of the 16S rRNA genes in each sample was amplified (KAPA HiFi HotStart Ready Mix; Kapa Biosciences Inc., Wilmington, MA), with primers 341F and 805R that contained a 5' overhang adapter sequence. The amplicon was purified using NucleoFast 96 PCR plates (Takara Bio Inc., Shiga, Japan). A second PCR was conducted to attach a unique combination of dual indices (I5 and I7) and Illumina Inc. sequencing adapters to each sample. After the amplicon of the second PCR was purified, the concentration was normalized using a kit (SequalPrep Normalization Plate Kit; Life Technologies Japan Ltd., Tokyo, Japan). Each of the normalized amplicons was then evenly pooled and concentrated using AMPure XP beads (Beckman Coulter Inc., Tokyo, Japan). From the library, 11 pM were combined with phiX Control (ver. 3, Illumina; expected 20%) and were sequenced using a 300 bp paired-end strategy on the sequencer (MiSeq; Illumina), according to the manufacturer's instructions.

Sequence data analysis. Post-processing sequencing data were analyzed using USEARCH ver. 8.0 and QIIME ver. 1.9.0 software, as described in earlier reports.[17–19] Clean Fastq data were aligned into operational taxonomic units (OTUs) at 97% similarity using open reference OTU picking against the 16S database (Greengenes database ver. 13.8).[20] Taxonomic identification was performed at the phylum and genus levels. The observed Chao1 and Shannon phylogenetic diversity indices were calculated using the R "phyloseq" package.[21] Principal coordinate analysis (PCoA) was applied to plot the variation in the unweighted and weighted UniFrac distances between samples.

Measurement of myeloperoxidase activity. Tissue-associated myeloperoxidase (MPO) activity, which is an index of tissue-associated neutrophil accumulation, was found using a modified method of Grisham *et al.*, as described in earlier reports.[22,23] The MPO activity was assessed by measuring the H_2O_2-dependent oxidation of 3,3',5,5'-tetramethylbenzidine. One unit of enzyme activity was defined as the amount of MPO that caused a change in 1.0/min absorbance at 460 nm and 37°C.

Immunosorbent analyses. Colonic mucosal homogenates were centrifuged at 15,000 rpm at 4°C for 15 min with subsequent harvesting of the resultant supernatants. The tumor necrosis factor-α (TNF-α) concentrations in these supernatants were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Biolegend, San Diego, CA) specific for mouse TNF-α. Assays were performed according to the manufacturer's instructions. After color development, optimal densities were measured at 450 nm using a plate reader (Infinite M200).

Statistical analysis. Differences between two groups were analyzed using Mann–Whitney's U test. Differences among three or more groups were analyzed directly using Tukey's multiple comparison test. Statistical analyses were conducted using statistical software (GraphPad Prism ver. 8.01; GraphPad Software Inc., La Jolla, CA). All results are expressed as means ± SD. Differences for which $*p<0.05$, $**p<0.01$, and $***p<0.001$ were inferred as statistically significant.

Results

Effects of yacon intake on the intestinal environment. Six-week-old mice were fed YD for 30 days. Yacon intake had no effect on body weight gain (Table 2). However, the cecal content weights, a marker of fermentation, were significantly higher in YD-received mice than in CD-received mice, whereas the pH values were lower in YD-received mice, meaning that the microbial fermentation of unavailable carbohydrate is accelerated in the cecum of the mice.

Next, we calculated the concentrations of cecal organic acids as metabolites of microbiota using HPLC system. Mice treated with YD produced significantly more succinic acid, lactic acid, acetic acid, and propionic acid than control mice did (Fig. 1). No difference was found in the production of formic acid, isobutyric acid, and butyric acid.

Effects of yacon intake on the intestinal mucosal barrier. Goblet cells are columnar epithelial cells characterized by secretion of mucins. Then they function as an intestinal barrier. To evaluate yacon intake effects on intestinal mucosal barrier, the histological features of the colonic mucus were quantified using AB/PAS staining. Figure 2A shows that distinct blue-staining mucus-producing goblet cells were increased clearly in the colon derived from YD-received mice. We also determined the fecal mucin content, which can reflect the degrees of intestinal mucus. The fecal mucin contents were significantly higher in YD-received mice than in CD-received mice, which were consistent with data obtained from AB/PAS staining (Fig. 2B).

Effects of yacon intake on gut microbiota. Diet strongly affects the composition and activity of the gut microbiota.[24] To

Table 2. Yacon intake effects on physical parameters at 30 days of treatment

|	CD	YD
Initial body weight (g)	18.9 ± 1.4	18.6 ± 0.9
Final body wight (g)	25.2 ± 1.3	25.6 ± 1.8
Body weight gain (g)	6.2 ± 1.2	7.2 ± 1.2
Cecal content (mg/mouse)	111.7 ± 26.7	293.1 ± 50.4***
Cecal pH	7.7 ± 0.3	7.4 ± 0.2*

C57BL/6J mice were fed with either an AIN-76A-based control diet (CD) or an AIN-76A supplemented with 10% (w/w) yacon sample (YD) for 30 days. Values are expressed as the mean ± SD of 9 or 11 mice in each group, and *$p < 0.05$, ***$p < 0.001$ compared to CD group.
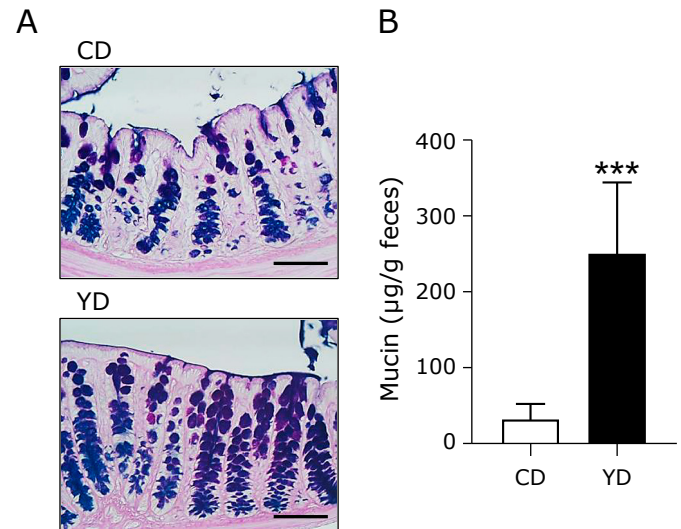
Fig. 2. Effect of yacon administration on fecal mucin content. (A) Representative images of alcian blue/periodic acid Schiff staining of the colon are shown (×40). Scale bar = 100 µm. (B) Concentration of fecal mucin was found using a specific fluorimetric assay. Data represent the mean ± SD of 9 or 11 mice; ***$p < 0.001$ was in comparison to CD-received mice.
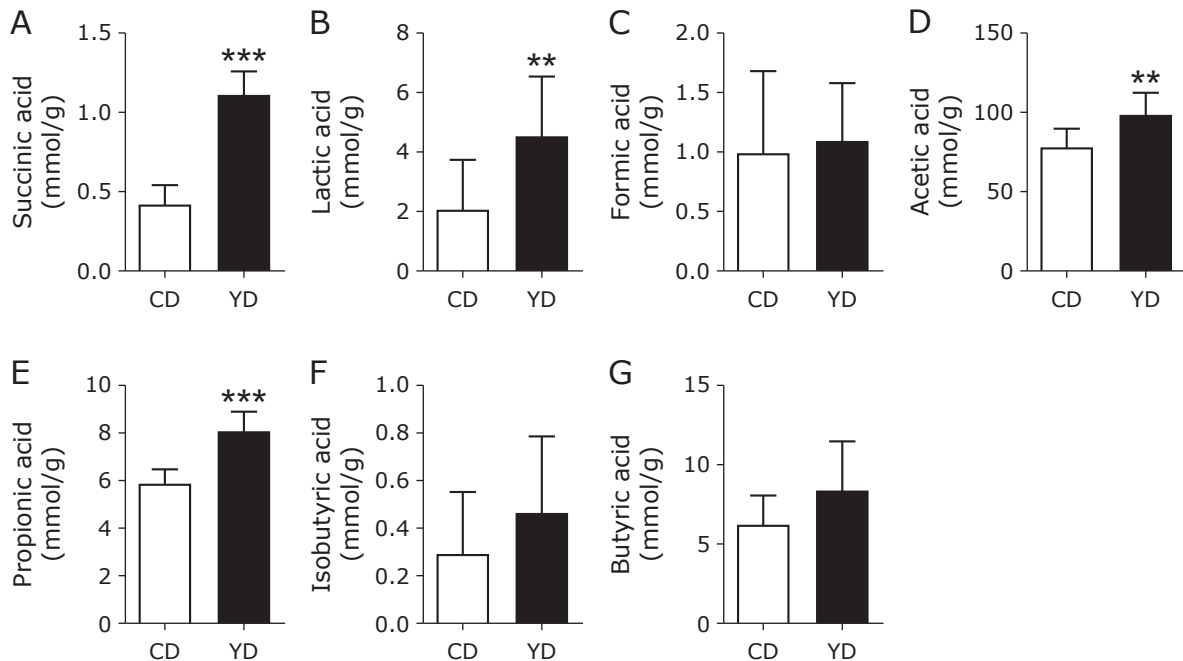
Fig. 1. Effects of yacon administration on short-chain fatty acids in mouse cecal contents. Concentrations of succinic acid (A), lactic acid (B), formic acid (C), acetic acid (D), propionic acid (E), isobutyric acid (F), and butyric acid (G) in the cecal are shown. Data represent the mean ± SD of 9 or 11 mice; **$p < 0.01$, ***$p < 0.001$ were in comparison to CD-received mice.
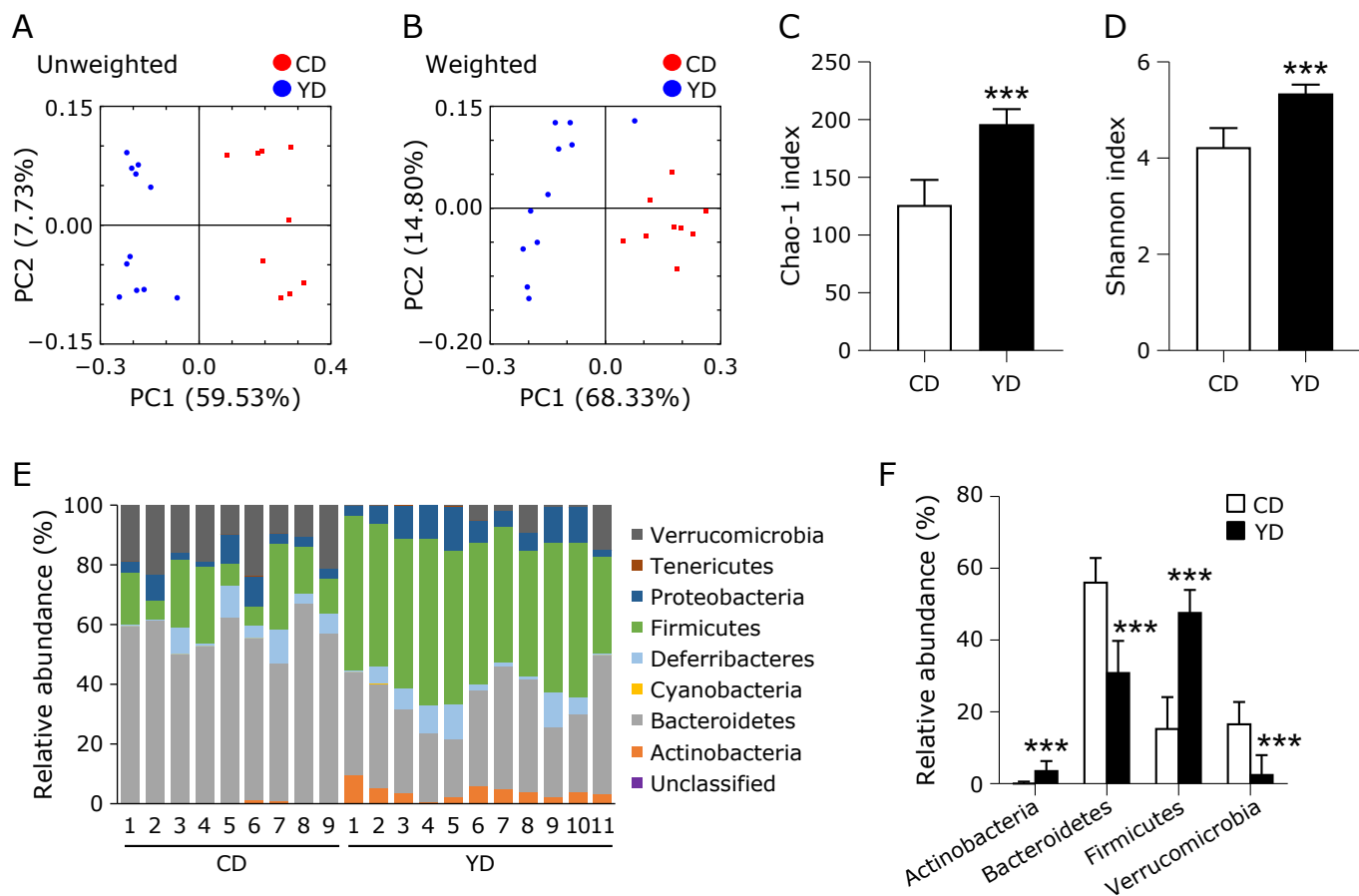
Fig. 3. Modulation of yacon administration on gut microbiota composition. (A, B) Principal coordinate analysis (PCoA) of unweighted and weighted UniFrac distances of microbial 16S rRNA sequences from the V3–V4 region in the stool samples. (C, D) Estimation of microbial community richness (Chao 1 index; OTU richness estimation) and diversity (Shannon index; OTU evenness estimation) for each sample. (E, F) Phylum level taxonomic distributions of the microbial communities in stool samples as ascertained from next-generation sequencing. The bar graph portrays significantly different taxa. Data represent the mean ± SD of 9 or 11 mice; ***$p < 0.001$ was in comparison to CD-received mice.

assess changes in the microbial community induced by yacon roots, variable regions V3–V4 of 16S rRNA genes extracted from the stool samples from the mice in each group were sequenced using Illumina MiSep platforms. The results are presented as OTUs using a 97% homology cutoff value. Initially, the overall structure of the gut microbiota using β-diversity indices were calculated for unweighted and weighted UniFrac distances. The YD-received mice exhibited a distinct microbiota composition clustered separately from that of the CD-received mice (Fig. 3A and B). Subsequently, we compared α-diversity among the CD-received and YD-received mice using different indices for the observed species and Chao 1 index (OTU richness estimation), and the Shannon index (OTU evenness estimation). Both indexes were increased in the YD-received mice compared to the CD-received mice (Fig. 3C and D).

For precise evaluation of yacon intake effects on gut microbial composition, we compared the relative abundance of the entire detected taxa for the respective groups. At the phylum level, yacon intake led to significantly higher relative abundances of Actinobacteria and Firmicutes than in CD-received mice (Fig. 3E and F), whereas the relative abundances of Bacteroidetes and Verrucomicrobia were lower in YD-received mice. At the genus level, the YD-received mice's microbial community showed significantly greater abundance of 22 genera, as presented by *Bifidobacterium*, *Lactobacillus*, *Oscillospira*, and *Allobaculum* (Table 3). The relative abundances of *Bacteroides*, *S24-7*, *Sutterella*, *Desulfovibrio*, and *Akkermansia* were lower in YD-

received mice (Table 4).

Preventive effects of yacon intake on TNBS-induced colitis. We examined TNBS-induced intestinal inflammation in the present mouse model to ascertain whether yacon-mediated modulation of intestinal environment including mucus secretion can suppress colitis, as shown in the experimental schedule in Fig. 4A. Macroscopic findings and scorings showed colitis with hyperemia, edema, thickening, and ulceration in TNBS-CD mice. These colitic features were suppressed in TNBS-YD mice (Fig. 4B and C). Representative histological images of vehicle-treated and TNBS-treated mice are depicted in Fig. 4D. Histological features confirmed the yacon intake-mediated suppression of colitis. Furthermore, tissue-associated MPO activity was measured in colonic tissue homogenates. Compared to that in the Sham-CD mice, MPO activity was significantly higher after TNBS treatment. The yacon intake significantly inhibited the increase in MPO activity (Fig. 4E). In the sham groups, yacon intake had no effect on the MPO activity. To analyze the effect of yacon intake further, we determined the expression level of TNF-α in the colonic tissue. Comparison to the sham groups showed that TNBS treatment significantly increased the TNF-α expression. The induction was suppressed by yacon intake (Fig. 4F).

Discussion

Results of this study show that oral administration of yacon

Table 3. Modulation of yacon intake on the composition of gut microbiota at the genus levels (*increased*).

Phylum	Class	Order	Family	Genus	CD	YD
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*	0.18 ± 0.30	3.40 ± 1.93***
Bacteroidetes	Bacteroidia	Bacteroidales	Unclassified	Unclassified	0.08 ± 0.07	3.09 ± 1.84***
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	*Parabacteroides*	0.02 ± 0.03	0.15 ± 0.07***
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified	1.05 ± 0.69	2.38 ± 0.98**
Firmicutes	Bacilli	Gemellales	Gemellaceae	*Gemella*	0.00 ± 0.00	0.01 ± 0.01*
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	0.04 ± 0.03	4.38 ± 3.99**
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Lactococcus*	1.17 ± 0.61	2.76 ± 1.73*
Firmicutes	Clostridia	Clostridiales	Unclassified	Unclassified	1.20 ± 1.04	11.58 ± 4.83***
Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Unclassified	0.00 ± 0.00	0.08 ± 0.05***
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Unclassified	0.00 ± 0.00	0.01 ± 0.01*
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridium*	0.01 ± 0.01	0.82 ± 0.67**
Firmicutes	Clostridia	Clostridiales	Dehalobacteriaceae	*Dehalobacterium*	0.03 ± 0.06	0.32 ± 0.13***
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Coprococcus*	0.01 ± 0.01	0.91 ± 1.14*
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[*Ruminococcus*]	0.14 ± 0.26	0.77 ± 0.49**
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Unclassified	0.00 ± 0.00	0.14 ± 0.09***
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unclassified	0.03 ± 0.03	1.56 ± 0.89***
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Oscillospira*	1.86 ± 1.92	6.24 ± 2.91**
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcus*	0.09 ± 0.06	0.84 ± 0.40***
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Unclassified	0.00 ± 0.01	0.12 ± 0.06***
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	*Allobaculum*	0.77 ± 1.41	5.02 ± 2.80***
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Unclassified	0.76 ± 1.05	7.60 ± 4.09***
Tenericutes	Mollicutes	RF39	Unclassified	Unclassified	0.00 ± 0.00	0.03 ± 0.03**

C57BL/6J mice were fed with either an AIN-76A-based control diet (CD) or an AIN-76A supplemented with 10% (w/w) yacon sample (YD) for 30 days. Values are expressed as the mean ± SD of 9 or 11 mice in each group, and *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to CD group.

Table 4. Modulation of yacon intake on gut microbiota composition at the genus level (*decreased*)

| Phylum	Class	Order	Family	Genus	CD	YD
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	15.26 ± 4.60	7.11 ± 2.43**
Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	Unclassified	37.86 ± 9.25	16.72 ± 4.71***
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Sutterella*	3.36 ± 2.68	0.31 ± 0.47*
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	*Desulfovibrio*	0.57 ± 0.24	0.34 ± 0.18*
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	*Akkermansia*	17.02 ± 5.41	3.05 ± 4.71***

C57BL/6J mice were fed with either an AIN-76A-based control diet (CD) or an AIN-76A supplemented with 10% (w/w) yacon sample (YD) for 30 days. Values are expressed as the mean ± SD of 9 or 11 mice in each group, and *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to CD group.

roots is associated with increased weight and SCFA levels, and decreased pH in the cecal contents, indicating that yacon roots can facilitate intestinal fermentation. Furthermore, results demonstrated that yacon intake suppressed TNBS-induced colitis in mice, which is associated with significant suppression of neutrophil accumulation and TNF-α production.

Indigestible carbohydrates, including oligosaccharides, were fermented and used by microbiota without degradation by digestive enzymes of the host. Consequently, administration of indigestible carbohydrates affects the gut microbial composition. Indeed, the relative abundances of 22 taxa were significantly higher in YD-received mice than in control mice. Of these, *Bifidobacterium pseudolongum* (the closest species of *Bifidobacterium*) and *Lactobacillus reuteri* (*L. reuteri*; one of the closest species of *Lactobacillus*) produce several SCFAs including lactic acid, acetic acid, and propionic acid as metabolites from several substrates such as glycerol and glucose.[25,26] As reported by Hopkins *et al.*,[27] *Bifidobacterium pseudolongum*

showed higher specific growth rates and bacterial cell yields than other species of *Bifidobacterium* in a medium with FOS as the sole carbon source. It is particularly interesting that *L. reuteri* produces succinic acid from citric acid and related acids.[28] Data from the SCFAs concentrations show that the succinic acid, lactic acid, acetic acid, and propionic acid concentrations were significantly higher in YD-received mice. These SCFAs increases might have been associated with the increases of the *Bifidobacterium pseudolongum* and *L. reuteri*.

Results of this study show that the fecal mucin contents, which can reflect the degrees of intestinal mucus, were significantly higher in YD-received mice than in CD-received mice. Recent evidence has demonstrated that the gut microbiota and its metabolite SCFAs can influence the properties of the intestinal mucus layer.[29,30] Burger-van Paassen *et al.*[31] reported that some SCFAs including acetic acid and propionic acid increase MUC2 expression, which is a major component of intestinal mucus, in human goblet cell-like LS174T cells. With regard to the micro-
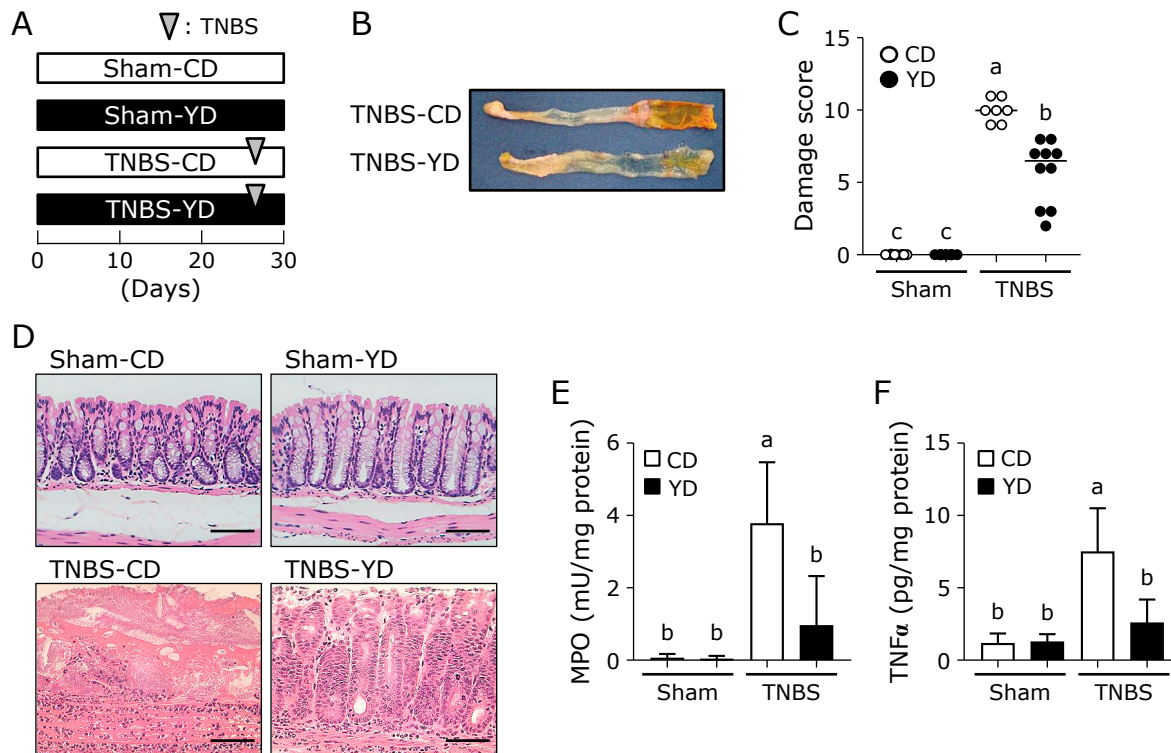
Fig. 4. Effect of yacon administration on intestinal inflammation. (A) Schematic representation of the experimental schedule for individual mouse groups. Gray arrowheads denote the TNBS treatment. (B) Macroscopic findings in the colon from the mice of TNBS-treated groups are presented. (C) Damage score of TNBS-induced colitis was evaluated as described in Materials and Methods. Data are expressed as a scatter plot. The bar shows mean values. (D) Histological appearances of the colon are shown. All sections were stained with hematoxylin and eosin (×40). Scale bar = 100 μm. (E, F) Tissue-associated MPO activity and the protein content of TNF-α were determined in the colonic mucosa. Data represent the mean ± SD of 7–10 mice. Statistically significant differences ($p < 0.05$) are denoted by different letters.

biota, the relative abundances of five taxa were significantly lower in YD-received mice than in control mice. Of these, *Akkermansia muciniphila* (the closest species of *Akkermansia*) was significantly lower in YD-received mice. Earlier reports have described that polyphenols from cranberry, apple, and green tea increased the relative abundance of *Akkermansia*.[32–34] Several indigestible carbohydrates decreased the relative abundance of *Akkermansia*.[5,35] These findings are consistent with our obtained data. *Akkermansia muciniphila* is an intestinal mucin-degrading bacterium,[36] indicating that the abundance of the bacterium affects the amount of mucus. In fact, the relative abundance of *Akkermansia* is correlated negatively with fecal mucin contents ($r = -0.77$). Taken together, changes of SCFAs levels and relative abundance of *Akkermansia* may be associated with increased intestinal mucus in response to yacon intake.

The intestinal mucus layer forms a physical barrier between the potential toxic and noxious agents present in the gut lumen and the underlying tissues. Histologic analysis for patients with IBD often shows depletion of goblet cells in the colonic epithelium.[37] Therefore, we evaluated the effect of yacon intake on colonic mucosal injury based on the macroscopic damage score and histology using murine TNBS model, a well-known experimental model of IBD. TNBS treatment induced colonic injury with hyperemia, edema, thickening, and ulceration. Then, these colitic features were suppressed by yacon intake. Results of several studies have shown the relationship between TNBS colitis and gut microbiota.[38–40] Hrdý *et al.*[41] reported that *L. reuteri*-primed dendritic cells robustly promoted the differentiation of regulatory T cells, resulting in suppression of intestinal inflammation. A recent study demonstrated that, in addition to the bacterial components, secreted factors from *L. reuteri* were able to modulate dendritic cells function.[42] There results suggest that increases of intestinal mucus and relative abundance of *L. reuteri* associate with yacon-mediated suppression of colitis.

Results demonstrate that intake of yacon roots suppressed TNF-α production in colonic mucosa significantly, concomitant with inhibition of TNBS-induced MPO activity. TNF-α, a typical cytokine that is deeply associated with IBD pathogenesis, is mainly produced by macrophages and neutrophils. For treating IBD worldwide, TNF-α blocking agents have been used as therapeutic agents.[43] Nevertheless, an aqueous extract of yacon roots had no effect on TNF-α expression in lipopolysaccharides (LPS)-stimulated Raw264.7 cells (our unpublished data), suggesting that the inhibitory effect of TNF-α production by yacon is an indirect effect via metabolites such as SCFAs. Several reports have described that some SCFAs including acetic acid and propionic acid suppressed TNF-α production in LPS-stimulated neutrophils.[44,45] Vinolo *et al.*[45] also demonstrated that propionic acid diminished the LPS-stimulated TNF-α release through the inhibition of NF-κB activation and histone deacetylase activity. Furthermore, Chang *et al.*[46] reported that butyric acid and propionic acid modulated the inflammatory status of intestinal macrophages. Meanwhile, elevated succinic acid level within the gut lumen have reported in patients with IBD and animal models of intestinal inflammation, and the accumulated succinic acid can activate immune cells and enhance inflammation.[47] These findings are inconsistent with our obtained data. We believe that the pro-inflammatory effect of succinic acid is masked by elevated levels of acetic acid and propionic acid. Consequently, yacon-mediated suppression of intestinal inflammation might be related to increased SCFA levels.

In conclusion, results presented herein indicate that adminis-

tration of yacon modulates the intestinal environment including microbial composition, SCFA level, and mucus content. It then prevents TNBS-induced colitis in mice. However, this study did not clarify whether the gut microbiota and its metabolites, derived from YD-received mice, play a role in this anti-colitic effect. To support a more definitive role for the intestinal environment, further investigations using fecal transplantation must be conducted. Accumulating evidence demonstrates that the properties of intestinal mucus layer play a crucially important role in several lifestyle-related diseases such as colorectal cancer and diabetes. Additionally, Betge *et al.*[48] reported loss of MUC2 expression as a predictor of adverse outcomes in patients with colorectal cancer. Taken together, the data presented herein demonstrate the possibility that oral intake of yacon roots prevents various lifestyle-related diseases.

Author Contributions

The respective roles of the authors are the following: YH, KM, TE, and YN conceived the project and prepared the manuscript; YH, MH, HN, EN, KM, and TT performed the experiments; RI analyzed the sequence data. All authors critically reviewed the manuscript. YH supervised all aspects of the study.

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Abbreviations

AB	alcian blue
CD	control diet
ELISA	enzyme-linked immunosorbent assay
FOS	fructooligosaccharides
HE	hematoxylin/eosin
HPLC	high performance liquid chromatography
IBD	inflammatory bowel disease
LPS	lipopolysaccharides
L. reuteri	*Lactobacillus reuteri*
MPO	myeloperoxidase
OTU	operational taxonomic unit
PAS	periodic acid Schiff
PCoA	principal coordinate analysis
SCFA	short-chain fatty acid
TNBS	2,4,6-trinitrobenzene sulfonic acid
TNF-α	tumor necrosis factor-α
YD	yacon-containing diet

Conflict of Interest

YN received scholarship funds from EA Pharma Co., Ltd.; a collaboration research fund from Taiyo Kagaku Co., Ltd.; and received lecture fees by Mylan EPD Co., Takeda Pharma. Co., Ltd., Mochida Pharma. Co., Ltd., EA Pharma Co., Ltd., Otsuka Pharma. Co., Ltd., and Miyarisan Pharma. Co., Ltd. The present research was partly supported by these funds. Neither the funding agency nor any outside organization has participated in the study design or have any competing interests. These pharmaceutical companies had final approval of the manuscript. The other authors have no conflicts of interest to disclose.

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