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Effects of *Undaria pinnatifida* and *Laminaria japonica* on Rat's Intestinal Microbiota and Metabolite

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Abstract

This study examined the effects on weight changes, intestinal microorganisms, and production of short chain fatty acids (SCFAs) in rats following the consumption of *Undaria pinnatifida* (*U. pinnatifida*) and *Laminaria japonica* (*L. japonica*) and *in vitro* fermentation by intestinal microbiota. Forty-eight Sprague-Dawley rats aged four weeks were divided in to a basal diet group (control), a basal diet+10% dried *U. pinnatifida* group (BDUP), and a basal diet+10% dried *L. japonica* group (BDLJ) and subjected to a four-week feeding trial. The rat weights showed smaller increases after four weeks for the BDUP and BDLJ groups when compared to the control. The intestinal microorganisms through 16S ribosomal RNA (rRNA) profiling revealed distributions of Firmicutes in the intestinal microorganisms of 92, 72, and 78% in the control, BDUP, and BDLJ groups, respectively, while the distribution of Bacteroidetes were 4, 24, and 20, respectively. All 36 species of microorganisms that fall under *Prevotella*, *Alistipes*, and Bacteroides genera increased in number by at least four fold, whereas *Roseburia*, *Mollicute*, and *Oscillibacter* decreased more than half. Fifty-two species of microorganisms belonging to *Clostridium*, *Escherichia*, and *Enterobacter* genera classified as pathogenic microorganisms decreased in all the treatment groups when compared to the control groups. Implementation of *in vitro* intestinal fermentation gave larger butyric acid yields for the feeds containing *U. pinnatifida* and *L. japonica* when compared to the basal diet. These results indicate that the provision of *U. pinnatifida* and *L. japonica* changed the balance of the intestinal microbiota in rats, thereby suppressing weight gain while promoting butyric acid production in the large intestine.

Keywords: *U. pinnatifida*, *L. japonica*, Intestinal microbiota, Butyric acid, 16S rRNA

Introduction

In the intestines, microorganisms convert dietary fibers like alginic acid into SCFAs such as butyric acid, acetic acid, and propionic acid through fermentation processes to produce extra energy [1]. Butyric acid is used energy for intestinal epithelial cells and the remaining SCFAs are absorbed into the blood stream. SCFAs, including butyric acid, promote lipolysis of fat cells [2], regulate gut hormones, and suppress fat accumulation triggered by insulin [3,4].

Obesity with associated metabolic disorders has become a major threat in improving the average life span [5]. The obesity, a type of metabolic syndrome, is caused mainly by energy, and nutrient imbalances, and insufficient physical exercise, but may also be a result of genetic factors, hormonal diseases, medication, stress, and microbial infection [6-11]. Dietary fiber intake is recommended for the prevention and treatment of obesity. One source of fiber is seaweeds, such as *U. pinnatifida* and *L. japonica*, which have been used as food sources for centuries in East Asian countries, including Korea, Japan, and China. These two seaweeds contain alginic acid, a water-soluble dietary fiber with high physiological activity, at levels of 47.2% (*U. pinnatifida*) and 50.7% (*L. japonica*) of the dry weight, giving them the highest dietary fiber contents among vegetables and seaweed [12].

The microorganisms in the intestines are commonly represented by limited phylogenetic types, with only a few dominant species, and the formation of these specific microbiotas vary from individual to individual [13,14]. The dominant species in the microbiota of the large intestine are mostly a result of diet but can also be affected by age and antibiotics [15-18]. Interestingly, transplantation of the intestinal microbiota of mice made obese by feeding regulation to germ-free mice led to obesity and metabolic syndrome in the transplanted mice, which showed marked increases in fat accumulation [19]. Recent evidence now points to the Firmicutes and Bacteroidetes phyla, which form dominant groups in human intestinal microbiotas, as determinants of obesity: humans show obese or slim body types depending on the ratios of these two phyla. Increases in the ratio of Firmicutes promote obesity while increases in the ratio of Bacteroidetes make the person slim [20].

The present study examined the effects of consumption of *U. pinnatifida* and *L. japonica* on changes in the intestinal microbiotas of rats and especially on the production of SCFAs (butyric, acetic, and propionic acids) during *in vitro* intestinal fermentation. A second aim was to provide further clarification of the interrelationship between changes in intestinal microbiota following consumption of *U.*

pinnatifida and *L. japonica* and obesity and to identify the underlying mechanism.

Materials and Methods

Experimental animals

Forty-eight female Sprague-Dawley rats aged 4 weeks were bought from SMATAKO BioKorea (Osan, Korea) and acclimated for one week before use. Dried *U. pinnatifida* and *L. japonica* powders were purchased from Haeormbio Co. Ltd (Busan, Korea) and added to the animal feed as indicated. The animals were divided into three groups: control (C) group was fed the basal diet, the BDUP group was fed the basal diet+10% dried *U. pinnatifida*, and the BDLJ group was fed the basal diet+10% dried *L. japonica* (Table 1). The animals were divided into four repetitions of four animals per cage per group and were freely fed for four weeks and housed in the animal room at 20-25°C, humidity 40%-45%, and a day/night cycle of 12 hours. The rat body weights were measured at the beginning of the experiment and at intervals of one week thereafter. At the end of the study period, the animals were sacrificed and the contents of the large intestine and blood were collected. The blood was separated into plasma and serum and sent to the Green Cross Reference Lab. (Yongin-si, Korea) for determination of blood properties and chemical components. All procedures were approved by the Institutional Animal Care and Use Committees at Gyeongnam National University of Science and Technology (No. 2014-04).

Bacterial quantification by qPCR

The rats were anesthetized using ether to collect intestinal contents for extraction of genomic DNA of the intestinal microorganisms. The total fecal bacterial load was calculated by isolating the total bacterial DNA from weighed feces using a ZR Fecal DNA MiniPrep™ (Zymo Research, USA) according to the manufacturers. The extracted genomic DNA was sent to ChunLab (Seoul, Korea) for qPCR as follows. The extracted DNA was amplified using primers targeting the V1-V3 regions of the prokaryotic 16S rRNA gene. The primers used for bacteria were V1-9F (5'-CCTATCCCCTGTGTGCCTTGGCAGTC-TCAG-AC-GAGTTTGATCMTGGCTCAG-3') and V3-541R (5'-CCATCTCATCCCTGCGTGTCTCCG ACTCAG- barcode-AC-WTTACCGCGGCTGCTGG-3') [21]. The cycling conditions were an initial denaturation step at 94°C for 5 min, followed by 10 cycles of denaturation at 94°C for 30 s, annealing at 60°C to 55°C (with a touchdown program) for 45 s, and elongation at 72°C for 90 s, and a final elongation step at 72°C for 5 min. The sizes of amplicons were 500-700 bp for bacteria. The amplified products were purified using resin columns (Qiagen, Germany), and 1 μg of the PCR product from each sample was mixed and purified using the AMPure bead kit (Agencourt Bioscience, USA). The DNA was sequenced at ChunLab, Inc. with a Roche/454 FLX system, according to the manufacturer's instructions.

16S rRNA gene sequence analysis

The pyrosequencing data for the 16S rRNA gene sequences was processed through Java-based multi-step bioinformatics pipelines. The unidirectional sequencing reads were separated by their unique barcodes. Low quality sequences which were the reads <300 bp were filtered by omitting. The trimmed sequencing reads were assembled into sets of highly similar sequences a TBC clustering algorithm with a 97% cutoff [22]. Representative sequences in clusters of trimmed sequences were chosen for identification. Singletons were considered as individual operational taxonomic units (OTU). The representative sequences and singletons were assigned to taxonomic positions according to the highest pairwise similarity among the top five BLASTN hits against the EzTaxon-e database [23]. Sequences that showed no match in a BLASTN search (expectation value of $>e^{-5}$) against the EzTaxon-e database were considered to be non-target sequences and were ignored. The nucleotide sequence similarity between the query and the candidate species was calculated using the Myers and Miller global pairwise alignment along with CLUSTAL [24,25]. Potential bias, which was caused by different sequencing depths was avoided by rarifying samples with more the 3,000 reads to a depth of 3,000 reads for subsequent analysis. The diversity measures were calculated by using a TBC clustering algorithm, and the cutoff value for assigning a sequence to a species-level OTU was ≥ 97% similarity. The overall phylogenetic distance between each pair of communities was estimated using Fast UniFrac analysis in the CLcommunity program.

Production of short chain fatty acids by *in vitro* intestinal fermentation

The contents of the colons of 11 week old female Sprague-Dawley rats were extracted to examine SCFA production by *in vitro* anaerobic culture of intestinal microorganisms. An anaerobic mineral salt medium (pH 6) was charged with CO_2 gas for one hour and the large intestinal contents and the mineral salt medium were mixed at a ratio of 1:9. This mixture was added to 2% of the rat diets (basal diet (Control), or basal diet containing dried *U. pinnatifida* (BDUP), or dried *L. japonica* (BDLJ)) in 60 ml bottles, sealed, and cultured sealed under anaerobic conditions at 38°C in a shaking incubator. One ml samples of culture supernatant collected after 0, 3, 6, 9, 12, and 24 hours of anaerobic culture and centrifuged for 10 minutes at 1,500 xg. The supernatants were filtered through 0.2 μm Millipore syringe filters and subjected to HPLC (Agilent Technology, Germany) using 300 × 7.8 mm MetaCard 87H columns (Varian, USA) to quantitatively identify SCFAs (acetic, butyric, and propionic acids).

Statistical analysis

The experimental results were statistically analyzed by conducting, analyses of variance using the General Linear Model of the SAS package program (V 9.1). The differences between processed means were analyzed by Tukey's multiple range tests at a 95% significance level.

Results

Growth efficiency and weight gain

The treatment groups (BDUP and BDLJ) showed smaller average daily weight gain, average daily feed, feed efficiency, and reduced feed intakes when compared to the control group (Table 1).

Items	Treatments				
	Control	BDUP	BDLJ	SEM	p-value
Initial body weight (g)	136.2	137.3	137.1	1.6	0.87

Finished body weight (g)	223.4[a]	207.8[b]	201.3[b]	3.8	<0.01
Average daily gain (g)	3.11[a]	2.52[b]	2.29[b]	0.14	<0.01
Average daily feed intake (g)	15.7[a]	14.5[b]	13.9[b]	0.3	<0.01
Feed efficiency	0.199[a]	0.173[ab]	0.165[b]	0.01	<0.01

Table 1: Growth efficiency and weight gain by feeding *U. pinnatifida* and *L. japonica* in rats. Control: basal diet, BDUP: basal diet+10% dried *U. pinnatifida*, BDLJ: basal diet+10% dried *L. japonica*. [a,b]Means with different superscripts in the same row are significantly different ($p<0.05$).

No significant difference was noted among the treatment groups. Blood lipids (serum triglyceride and LDL- and HDL-cholesterol concentrations) measured after the 4 week feeding trial showed no differences between the control group and any of the treatment groups (data not shown). However, the recorded body weight gains were lower in the BDUP and BDLJ groups when compared to the control group at the end of the four week feeding trial.

Classification of intestinal microorganisms according to sample treatments

The control group contained 429 species of microorganisms, while the BDUP group and the BDLJ contained 399 and 450 species, respectively (Table 2).

Classification	Control	BDUP	BDLJ
Phylum	8	8	8
Class	13	13	12
Order	19	22	23
Family	52	54	60
Genus	199	189	216
Species	429	399	450

Table 2: Analysis of the gut bacterial community by 16S rRNA pyrosequencing from rat fed different seaweeds. Control: basal diet, BDUP: basal diet+10% dried *U. pinnatifida*, BDLJ: basal diet+10% dried *L. japonica*. The values are the number of the phylotypes in each taxon level.

The distribution ratios of intestinal microorganisms revealed that Firmicutes and Bacteroidetes accounted for at least 96% of the microorganisms in all groups, including the control group, thereby representing the dominant bacteria among intestinal microorganisms (Figure 1). Firmicutes decreased from 92% in the control group to 72% in the BDUP and 78% in the BDLJ groups. On the other hand, the distribution of Bacteroidetes was 4% in the control group and increased to 24% in the BDUP and 20% in the BDLJ groups (Figure 1A). Thus, the addition of either of the seaweeds or a mixture of *L. japonica* decreased the Firmicutes/Bacteroidetes ratio in the intestine to a degree proportional to the decrease in weight gains. As shown in Figure 1B, the BDUP and the BDLJB groups showed the difference in distribution of intestinal microorganisms from the control group.

The distributions of intestinal microorganisms were also analyzed at the genus level by function to identify anti-obesity (lean), obesity, pathogenic, and butyric acid-producing bacteria (Table 3).

	Control	BDUP	BDLJ
Lean-related microbes			
Prevotella (7)[1]	0.002	2.2	2.02
Alistipes (9)			
Bacteroides (20)			
Obesity-related microbes			
Roseburia (6)	0	-1.84	-1.4
Mollicute (14)			
Oscillibacter (15)			
Pathogenic microbes			
Clostridium (49)	0	-1.4	-1.32
Escherichia coli (1)			
Enterobacter (2)			
Butyric acid-generated microbes			
Roseburia (6)	0	-0.38	1.24
Butyrivibrio (2)			
Eubacterium (6)			
Coprococcus (1)			
Anaerotruncus colihominis (1)			
Butyricicoccus (1)			

Table 3: Genus-level analysis of different functional microorganisms upon different seaweed administration in rat's intestine. Control: basal diet, BDUP: basal diet+10% dried *U. pinnatifida*, BDLJ: basal diet+10% dried *L. japonica*. [1]Numbers of species-level microorganisms belong to each genus. [2]All values are the $\log_2$ transformed values which sum up the ratio of read numbers of 16S rRNA pyrosequencing.

The *Prevotella*, *Alistipes*, and Bacteroides genera, which are known anti-obesity (lean) related micro-organisms, were present as 36 species-level phylotypes in the intestines of control animals. The distributions of these genera changed in response to seaweed consumption, increasing at least four fold in the BDUP and BDLJ groups compared to the control group. The *Roseburia*, *Mollicute*, and *Oscillibacter* genera, which are microorganisms that show high distributions in the intestine of obese individual, were present as 35 species-level phylotypes in the control group. The distributions decreased to 28% in the BDUP group and 38% in the BDLJ group. The intestines of the control group also contained 52 species-level phylotypes of microorganisms belonging to the *Clostridium*, *Escherichia*, and *Enterobacter* genera, including many pathogenic bacteria. Compared to the control group, the distributions of these three genera decreased to 38% in the BDUP group and 40% in the BDLJ group. The control group also contained 17 species-level phylotypes of microorganisms belonging to *Roseburia*, *Butyrivibrio*,

Eubacterium, *Coprococcus*, *Anaerobruncus colihominis*, and *Butyricicoccus*, which are microorganisms known to hydrolyze indigestible dietary fibers in the large intestine to produce the SCFA butyric acid. Compared to the control group, the distribution of these six genera slightly decreased in the BDUP group but increased by at least two fold in the BDLJ group.

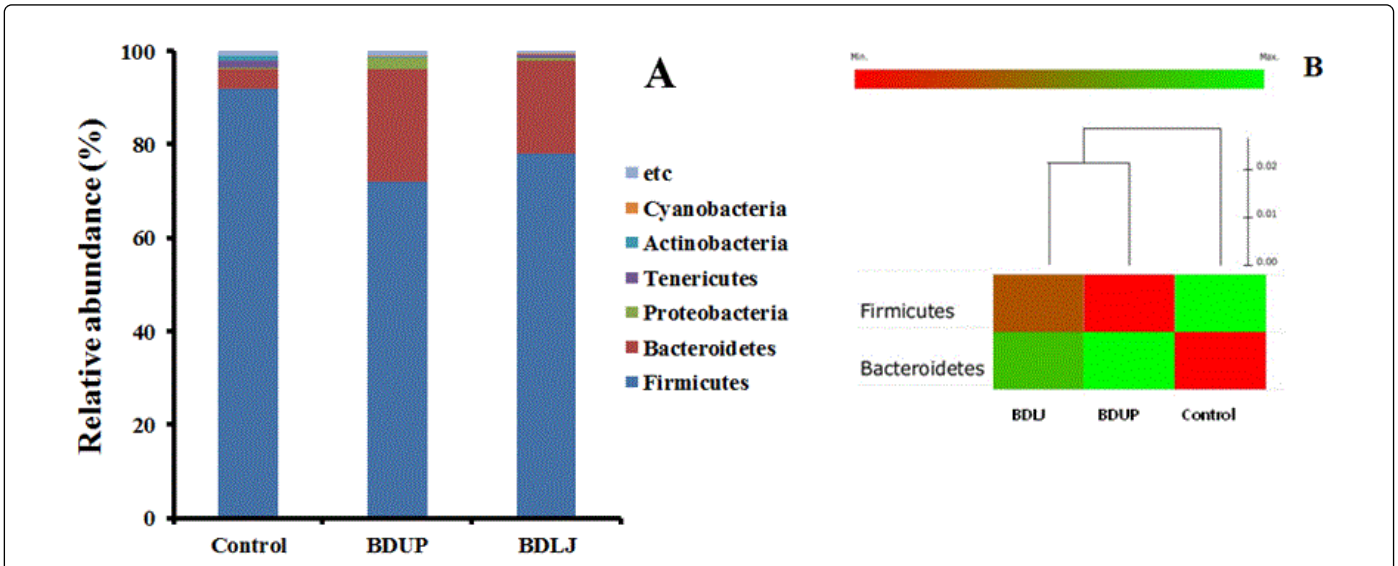

Figure 1: Phylum-level distributions of intestinal microbiota of rats fed diets containing different seaweeds, and their heat map. A: Phylum-level distribution of intestinal microorganisms. B: the heat map of each group. Control: basal diet, BDUP: basal diet + 10% dried *U. pinnatifida*, BDLJ: basal diet + 10% dried *L. japonica*.

Short chain fatty acid production from *U. pinnatifida* and *L. japonica* through *in vitro* intestinal fermentation

The yields of the three SCFAs from the different rat diets increased with proceeding in fermentation time from 0 hour to 24 hours. Among the three fatty acids, the yield of butyric acid was the highest (Table 4). The yields of acetic acid and propionic acid increased more in the basal diet compared to the BDUP and BDLJ feeds over time and the differences were after nine hours of anaerobic culture. Greater amounts of butyric acid were produced from the BDUP and BDLJ feeds compared to the basal diet. The feed containing *U. pinnatifida* produced the largest amount of butyric acid after six hours of anaerobic culture.

Time (h)	Treatment[a]		
	Control	BDUP	BDLJ
Butyric acid (ppm)			
0	2113 ± 100.1^{a}	2968 ± 34.3^{b}	3097 ± 56.1^{b}
3	2285 ± 153.2^{a}	2690 ± 94.6^{b}	3177 ± 42.3^{c}
6	2400 ± 91.0^{a}	3534 ± 77.8^{b}	3194 ± 19.5^{c}
9	2390 ± 186.4^{a}	3978 ± 118.4^{b}	3505 ± 71.7^{c}
12	2553 ± 315.2^{a}	4237 ± 127.0^{b}	3451 ± 189.1^{c}
24	2677 ± 170.4^{a}	5548 ± 76.5^{b}	4335 ± 41.2^{c}
Acetic acid (ppm)			
0	1102 ± 13.5^{a}	780 ± 127.6^{b}	1102a ± 11.3^{a}
3	1240 ± 8.9	1212 ± 9.2	1305 ± 118.5
6	1304 ± 8.3	1289 ± 72.3	1317 ± 95.6
9	1708 ± 5.25^{a}	1342 ± 33.4^{b}	1542 ± 11.1^{c}
12	1817 ± 112.5^{a}	1430 ± 43.8^{b}	1660 ± 13.0^{a}
24	1849 ± 94.4^{a}	1670 ± 19.3^{b}	1710 ± 29.3^{ab}
Propionic acid (ppm)			
0	266 ± 4.3^{a}	279 ± 4.1^{ab}	309 ± 13.9^{b}
3	608 ± 4.0^{a}	594 ± 1.1^{b}	668 ± 1.4^{c}
6	680 ± 41.5^{a}	656 ± 6.4^{a}	756 ± 17.9^{b}
9	982 ± 18.2^{a}	689 ± 9.3^{b}	837 ± 48.8^{c}
12	1049 ± 21.8^{a}	747 ± 15.1^{b}	910 ± 20.2^{c}
24	1102 ± 66.5^{a}	766 ± 13.5^{b}	977 ± 35.0^{a}

Table 4: Generation of short-chain fatty acids by *in vitro* intestinal fermentation. Mean ± SE with different superscripts in the same row are significantly different ($p<0.05$). Control; basal diet, BDUP; basal diet+10% dried *U. pinnatifida*, BDLJ; basal diet + 10% dried *L. japonica*.

Discussion

The over-weight and obese populations are rapidly growing in advanced countries as well as in new economically developed countries, leading to increases in adult diseases such as diabetes and heart disease. These diseases are related to metabolic syndrome and can be prevented by dietary control [26,27] and dietary fibers play a major role in preventing overweight or obesity [28]. In addition, the

microorganisms living in the alimentary canal of mammals have now also been associated with obesity and metabolic disorders [11,13,20]. Many enteric bacteria excrete through feces, which may lead undesirable effects in health and environment [29-32]. The distributions of intestinal microorganisms can change due to various factors but they can be changed in a short time by food. In particular, dietary fibers change the distribution of the intestinal microorganisms that aggravate overweight and obesity, thereby helping to prevent or improve obesity and metabolic disorders [26,33,34]. The present study indicates that addition of *U. pinnatifida* and *L. japonica* animal feeds can change the distributions of intestinal microorganisms in favor of those that prevent or improve overweight and obesity in rats.

The use of *U. pinnatifida* and *L. japonica* as foods has a long history in Northeast Asia. They are mainly available in their dried states, and the total dietary fiber content is approximately 50% of the dry matter, which is the highest content among vegetables and seaweeds [12]. Consumption of these dietary fibers reduces blood cholesterol level [27], and suppresses weight gain [28]. The addition of *U. pinnatifida* and *L. japonica* to rat feeds at a ratio of 10% had no effect on blood cholesterol concentrations when compared to the basal diet (data not shown), but weight gains and feed efficiency decreased in rats fed the supplemented diets (Table 1). The lower feed efficiency in seaweed treatment groups indicates to be less weight gain even to have the same feed intake. Several hypotheses have been proposed for the mechanism by which dietary fibers suppress weight gain. Dietary fibers, and especially those that are water soluble and viscous, have the effects of increasing satiety, reducing food intake, and decreasing nutrient absorption in the small intestine [35]. In addition, changes in the kinds and quality of indigestible carbohydrate fiber in foods affect the types of intestinal microorganisms and the production of SCFAs, which are metabolic products of intestinal microorganisms. Butyric acid, in particular, suppresses fat accumulation [36].

The microbiotas in individual environments can be identified by 16S rRNA gene sequencing analysis. The distribution of intestinal microorganisms is most affected by foods consumed and is directly related to the health of the host. Through interactions with the host, intestinal microorganisms are involved in host nutrition, growth, metabolic processes, resistance to pathogenic microorganisms, and regulation of immune responses [19,37,38]. The isolation and pyrosequencing of genomic DNA from the intestinal contents of rats fed diets supplemented with *U. pinnatifida* and *L. japonica* in the present study confirmed that the diversity of intestinal microorganisms differed according to the diets provided. Animals provided with *L. japonica* showed the highest diversity of microorganisms, with 450 species-level phylotypes (Table 2). The distributions of Firmicutes and Bacteroidetes were not lower than 96% in all the treatment groups, indicating that these two phylum-level phylotypes were the dominant bacteria. The distributions of intestinal microorganisms were similar among the two treatment groups, regardless of the additive (Figure 1), with decreases noted in the Firmicutes and increases in the Bacteroidetes when compared to the rats fed the basal diet. The decrease in the Firmicutes/Bacteroidetes ratio was inversely proportional to the rat weight gains. Thus, supplementation of the basal rat diet with *U. pinnatifida* and *L. japonica* resulted in suppressed weight gain and reduced the intestinal Firmicutes/Bacteroidetes ratios.

A comparison of European children with children from the Burkina Faso region in Africa, who typically consume a vegetarian diet, indicated that the children in the Burkina Faso region had more Bacteroidetes in their intestinal microbiotas than did the European children. The children from Burkina Faso also had relatively higher distributions of Bacteroidetes including *Prevotella*, which can hydrolyze cellulose and xylan. This genus was rarely found in the intestines of European children [33]. Fat accumulation was also decreased in mice with high intestinal distribution rates of *Prevotella* [26]. *Prevotella*, *Alistipes*, and Bacteroides genera, which are also members of the Bacteroidetes, are obesity-suppressing intestinal microbiotas highly distributed in slim persons [39]. The present study recorded 36 species of microorganisms that belong to these three genera in the rat intestine. All 36 of intestinal species increased by at least four fold in rats fed diets supplemented with *U. pinnatifida* or *L. japonica* (Table 3). *Roseburia* is a genus that increased in the intestines of broiler chickens in response to improved weight gains [40]. *Mollicute* is genus that increased in the intestines of animals induced by diets to be obese [19]. *Oscillibacter* is an intestinal genus that increases in the intestines of animals induced by high fat diets to be obese. This microorganism induces mild inflammation and metabolic disorders that result in accumulation of fat in fat cells [41]. The levels of *Roseburia*, *Mollicute*, and *Oscillibacter* genera, which are highly distributed in the intestines of obese animals, decreased to approximately one half in the animals in the present study following consumption of diets containing *U. pinnatifida* or *L. japonica* (Table 3). The levels of microorganisms associated with obesity suppression increased, while those associated with obesity decreased in the rats provided with *U. pinnatifida* or *L. japonica*.

Many studies have reported that the immune system and metabolic disorders have a close correlation. Inflammatory signaling affects metabolic signaling pathways, as indicated by the promotion of obesity by mild-grade inflammations [42,43]. Among intestinal microorganisms, pathogenic microorganisms are a factor that causes mild-grade inflammations. High-fat diets increased the pathogenic microbiota of the intestine, which leads to expression of inflammatory substances such as cytokines through the induction of Toll-like receptor 4, while increasing the permeability of the intestines [44]. In addition, the endotoxins of intestinal pathogenic microorganisms also cause inflammation through the activity of macrophages [45]. These inflammations increase the expression of TNF-α and NF-κB and affect the secretion of insulin, adiponectin, leptin, and resist in, thereby promoting obesity in the host animal [15,42]. The present study identified 52 pathogenic microorganisms belonging to the *Clostridium*, *Escherichia*, and *Enterobacter* genera in the intestines of control rats, and the levels of these pathogenic microorganisms decreased in animals provided with *U. pinnatifida* or *L. japonica* (Table 3).

Dietary fibers are fermented by microorganisms in the large intestine to produce SCFAs including butyric, acetic, and propionic acids. The SCFAs suppress the fat accumulation signaling triggered by insulin by interacting with short-chain fatty acid receptor GPR43, relieving inflammation, and reducing fats, cholesterol, and triglycerides in the liver [4,46]. Approximately 70% of butyrate is used as an energy source by intestinal cells and the remainder is absorbed into the blood stream. The butyrate absorbed into the circulation shows anti-inflammatory actions and induces the production of glucagon-like peptide 1, which stimulates satiety [47,48]. Propionic acid has been reported to increase satiety and acetic acid reduces weight gain regardless of the suppression of food intakes [3]. In addition, although acetic acid is used for synthesis of fat and cholesterol in the liver through the activity of cytosolic acetyl S CoA synthetase, high concentrations of acetate increase the expression of AMP kinase in rat liver cells and suppress fat synthesis [49,50]. The

effects of acetic acid on obesity require further study. Taken together, the data from the present experiment and other study reports indicate that butyric acid has the strongest weight gain suppressing effect. Butyric acid is used by certain intestinal microorganisms to produce dietary fibers [51-54], and 17 species belonging to six such genera, including *Roseburia*, were identified in rats provided with *U. pinnatifida* or *L. japonica* (Table 3). As shown in Table 3, the distributions of butyric acid-producing bacteria increased only in the BDLJ group, i.e., only in rats supplemented with *L. japonica*.

The kinds and yields of SCFAs produced by the fermentation of indigestible polysaccharides by intestinal microorganisms vary with the kinds of indigestible polysaccharides [52-56]. As shown in Table 4, the yields of acetic acid and propionic acid were the highest in the basal feed following *in vitro* culture. The yields of butyric acid were higher with the BDUP feed and the BDLJ feed when compared with the basal feed. The BDUP feed showed the highest yield of butyrate after six hours of culture.

In conclusion, in the present study, supplementation of a basal rat diet with *U. pinnatifida* and *L. japonica* resulted in a reduction in weight gain in rats fed those diets. This reduction in weight gain is considered to be related to changes in intestinal microorganisms. The consumption of the supplemented diets increased the distribution of anti-obesity (lean)-related microorganisms and suppressed the proliferation of obesity-related microorganisms as well as reducing the distribution of intestinal pathogenic microorganisms that cause mild-grade inflammations. *In vitro* anaerobic culture of intestinal microorganisms from animals that consumed the different feeds indicated that the microbiotas from animals fed *U. pinnatifida* or *L. japonica* produced more butyric acid than the microbiotas from the basal diet group. Therefore, the intake of *U. pinnatifida* and *L. japonica* appears promising for preventing or improving overweight or obesity.

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