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Investigation by Microarray Analysis of the Immunostimulatory Function of an Extract of the Genus Plant Salacia in the Small Intestine of Rats

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Abstract

In our previous studies, Salacia extracts demonstrated beneficial effects on the enteric environment of the rat, as represented by decrease in ammonia and other products decomposed by enteric microorganism. In the present study, we showed that the expression of immunologically relevant genes increased in the epithelium of the small intestine from the rat orally dosed with Salacia extracts. T-RFLP analysis (Nagashima method) revealed altered composition of intestinal flora. Salacia extracts reportedly inhibit enzymatic degradation of polysaccharides, hence blocking the intestinal absorption of polysaccharides. These results taken together suggested that unabsorbed polysaccharides may affect the intestinal flora environment through the enteric immune system of the rat.

1. Introduction

It is known that extracts of plant in the genus Salacia, such as Salacia reticulata and Salacia oblonga, contain salacinol, kotalanol, mangiferin, catechin and many other components although not all the components those extracts contain are identified yet[1].

Some in-vitro experiments have proved that salacinol and kotalanol extracted from plant in the genus Salacia plants have been shown to exert an inhibitory effect *in vitro* on both α-glucosidase activity and blood glucose elevation in glucose-loaded rats[2]. It has been also proved that extracts of the genus Salacia improve conditions of diabetic patients and rat models of diabetes[3].

The small intestine, where α-glucosidase is secreted, is a vital organ. It takes in nutrients and eliminates foreign bodies by its immune function. It is considered to be the area where Salacia extracts work. But, it is yet to be determined how they work in the intestinal tract.

Another report says that catechin and mangiferin, also extracted from plant in the genus Salacia, have an antiobesity effect[4]. Other findings on the genus Salacia have been also reported[5-6]. However, most of the findings are effects on diabetes and obesity. There are few findings on other effects or the mechanism of function in a living body. The action of several components of a Salacia extract has not been defined.

This study aims at finding out the physiology of plant in the genus Salacia in the small intestine, which is the area where the extract inhibits sugar absorption and which has absorption, removal of foreign bodies and various other functions. In the experiment, we gave an extract of plant in the genus Salacia to rats and conducted gene expression analysis using a microarray and profiled the intestinal flora using T-RFLP. In the lower part of the small intestine, the administration of a Salacia extract accelerated expression of several immune-related genes, especially those relating to Th1, which contributes to cell immunity. In the large intestine, it increased bacteria that have an immune function. A Salacia extract caused changes in intestinal flora.

2. Experiments

2.1 Preparing an Extract Powder of Plant in the Genus Salacia

We used a species of plant in the genus Salacia (Salacia reticulata) that grew in Sri Lanka and dried the stem and root to make chips. The well-dried chips were left them in hot water for an hour and then filtered out. Resultant liquid was cooled to be reduced to powder using a spray dryer ADL-310 (Yamato Science Co., Ltd., Tokyo, Japan), and kept at 4 °C.

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2.2 Animal

We bought 6-week-old male Sprague Dawley® rats (SD rats) (CLEA Japan, Inc., Shizuoka, Japan) and kept them for one week for quarantine and habituation in the following environment: room temperature of 23 ± 2 °C, relative humidity of 50±10%, ventilation of 15 times per hour and artificial lighting for 12 hours a day. We gave the rats a solid feed sterilized with irradiation CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan), letting them eat it freely. For drinking water, we gave city water that complies with the water quality requirements by the Water Supply Act after filtering (50 μm and 5 μm) (AION Co., Ltd., Osaka, Japan) and sterilizing with UV radiation, through an automatic watering nozzle to let them drink freely. One week later, we divided the rats into two groups of 10 at random.

We dissolved the Salacia extract powder in an injection solvent (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) to make an 80 mg/ml solution. Using a metal gastric tube, we forced oral administration of the Salacia extract solution into the stomach of the test group until they were given 20 mg/kg in the weight of extract powder. To the control group, we gave the injection solvent only. The administration was repeated once a day for 13 weeks. We fasted the rats for 16 hours from the evening of the day of last administration. After anesthetizing the rats with pentobarbital sodium, we sampled the blood and euthanized them by exsanguinations. We dissected the rats, weighed the respective organs and observed the conditions. We took out the ileum, removed the epithelium and kept the epithelium in ISOGEN (NIPPON GENE Co., Ltd., Tokyo, Japan). We also sampled feces from the lower part of the large intestine and froze them with dry ice.

During the dissection, we also sampled blood from the posterior aorta and added an anticoagulant, EDTA-2K, to the blood to conduct biochemical test.

We tested the blood on these items: white blood cell count (WBC), red blood cell count (RBC), hemoglobin content (HGB), hematocrit level (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), reticulocyte count (Reti), prothrombin time (PT), activated partial thromboplastin time (APTT), total protein (TP), albumin level (ALB), A/G, triglyceride (TG), total cholesterol (T-CHO), blood urea nitrogen (BUN), creatine (Cre), Calcium (Ca), inorganic phosphorus (IP), AST activity (AST), ALT activity (ALT), CPK activity (CPK), total bilirubin (T-BIL), sodium (Na), potassium (K), and chlorine (Cl).

We measured WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT and Reti using a comprehensive hematology analyzer, XT-2000iV (Sysmex Co., Ltd., Hyogo, Japan). To measure PT and APTT, we used a fully automated coagulation and fibrinolysis analyzer, STA Compact (Roche Diagnostics K.K., Tokyo, Japan). We used an automatic biochemical blood analyzer, H7070 (Hitachi Ltd., Tokyo, Japan), for measurement of TP, ALB, A/G, Glu, TG, T-CHO, BUN, Cre, Ca, IP, AST, ALT, GGT, ALP, CPK, T-Bil, Na, K and Cl.

We verified the weights of the rats and their organs (absolute weights and relative weights) and biochemical test data of blood as below.

We first conducted F-test to test the equality of variance and then checked for significant differences using Student's t-test. The tissues were preserved and fixed in 10% neutral buffered formalin. We made specimen slices of the tissues, stained them with hematoxylin-eosin (HE) stain, and observed them with an optical microscope. We did not do verification concerning the general conditions, pathoanatomical test result or histopathological test result.

All of the animal experiments were approved by the Animal Care and Use Committee for Fujifilm.

2.3 RNA Extraction and DNA Microarray Analysis

From the preserved rat ileum cells, we extracted total RNA by the standard procedure using ISOGEN and then purified the total RNA using the RNeasy Mini Kit (QIAGEN, Hilden, Germany). We chose four rats with a weight closest to the average from each of the Salacia extract-treated group and the control group. From the total RNA of those rats' ilea, we synthesized cDNA, synthesized and labeled cRNA, and fragmented labeled cRNA, using an Affymetrix kit in accordance with the Affymetrix protocol. To check the quality of RNA, we used the Agilent 2100 bioanalyzer (Agilent Technologies Japan, Ltd., Tokyo, Japan) and confirmed that cRNA was elongated sufficiently. The fragmented cRNA was hybridized to GeneChip® Rat Genome 230 2.0 Array (Affymetrix Inc., CA, USA) at 45 °C for 16 hours in the Hybridization Oven 640 (Affymetrix Inc., CA, USA) and then washed and stained using the GeneChip® Fluidics Station 450. We scanned the cRNA with the GeneChip® Scanner 3000 and measured the gene expression levels. The acquired data were normalized in the Distribution Free Weighted (DFW) method using Bioconductor version 2.2 with the R version 2.7.2. We conducted Rank Products analysis to compare the results of the two groups and extracted a probe set with significant changes in gene expression that had a false discovery rate (FDR) below 0.05[7-11].

We classified the extracted probe set by biological function while referring to the Gene Ontology tool, Bingo 2.3 (Cytoscape 2.6) (http://www.psb.ugent.be/cbd/papers/BiNGO/index.htm) to draw a tree structure[12-13].

2.4 Analysis of Intestinal Flora (T-RFLP)

We outsourced the analysis of intestinal flora using the rat feces to TechnoSuruga Laboratory Co., Ltd., (Shizuoka, Japan). The analysis was conducted using T-RFLP (Nagashima method)[14]. The following are alterations made to the procedures in the reference literature.

The frozen feces were suspended in a GTC buffer (100 mM Tris-HCl [pH 9.0], 40 mM Tris-EDTA [pH 8.0] and 4M Guanidine Thiocyanate). The feces in the solution were lysed with zirconium beads (at 5 m/s for 5 minutes by the FastPrep FP100A Instrument (MP Biomedicals, CA, USA). From the 100-µl suspension, DNA was extracted using an automatic nucleic acid extractor (Precision System Science, Chiba, Japan). The reagent used for the automatic nucleic acid extraction was GC series Genomic DNA whole blood (Precision System Science, Chiba, Japan). The primer for the PCR, FAM-labeled 516F was used instead of HEX-labeled 516F as specified in the reference. The PCR products were purified with the MultiScreen PCRµ96 plate (Millipore, Billerica, MA, USA).

The fragment analysis was conducted with the ABI PRISM 3130x1 genetic analyzer (Applied Biosystems, CA, USA) using an analysis software, Gene mapper (Applied Biosystems, CA, USA). As a size standard marker, the MapMarker® X-Rhodamine Labeled 50-1000bp (BIOVENTURES, TN, USA) was used. Using a ratio of the peak area of each OTU (operational taxonomic unit) to the total peak area, hierarchical cluster analysis (using the pvclust function) was carried out to measure the similarity in flora pattern.

3. Results

3.1 Biochemical Test Results

No significant differences were found between the Salacia extract-treated group (20 mg/kg) and the control group after 13 weeks of continuous administration in weight (541.2 ± 47.8 g vs 578.2 ± 76.0 g) or biochemical blood test (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, Reti, TP, ALB, A/G, Glu, TG, T-CHO, BUN, Cre, Ca, IP, AST, ALT, GGT, ALP, CPK, T-Bil, Na, K and Cl). Concerning all the individual rats, we weighed the brain, pituitary, thymus, lung, liver, kidney, spleen, heart, adrenal gland, testicle, epididymis, seminal vesicle and prostate gland (ventral lobe). WE then fixed the liver in a 10% neutral buffered formalin, stained the specimen slices with hematoxylin-eosin, and observed them with an optical microscope.

As a result, we found no changes that indicated toxicity under the test conditions above.

3.2 Microarray Analysis

After we confirmed there are no findings indicating toxicity, we chose four rats with a weight closest to the average from each group and conducted the microarray analysis. We extracted a probe set of 237 genes with increased expression and 111 genes with decreased expression in the test group compared with the control group.

3.2.1 Genes with Increased Expression

The gene ontology result acquired by the analysis above shows that genes relating to oligopeptide transport, defense response, response to nutrient levels, antigen processing and presentation of peptide or polysaccharide antigen via MHC class II have been concentrated. Taking a close look at the genes with increased expression, many of them are genes relating to biological defense including immune-related genes, and genes relating to transport and metabolism (Fig. 1).

Among the MHC class II genes involved in antigens recognition, the genes with increased expression are Cathepsin E (Ctse), RT1 class II, locus Ba (RT1-Ba), HLA class II histocompatibility antigen, and DM beta chain precursor (MHC class II antigen DMb, Hla-dmb)[15].

Among the genes relating to biodefense (immunity), changes have been made in tumor necrosis factor alpha (Tnfα), Clusterin (Clu), Chemokine (C-C motif) ligand 5 (Ccl5, Rantes), Adenosine deaminase (Ada), Apolipoprotein

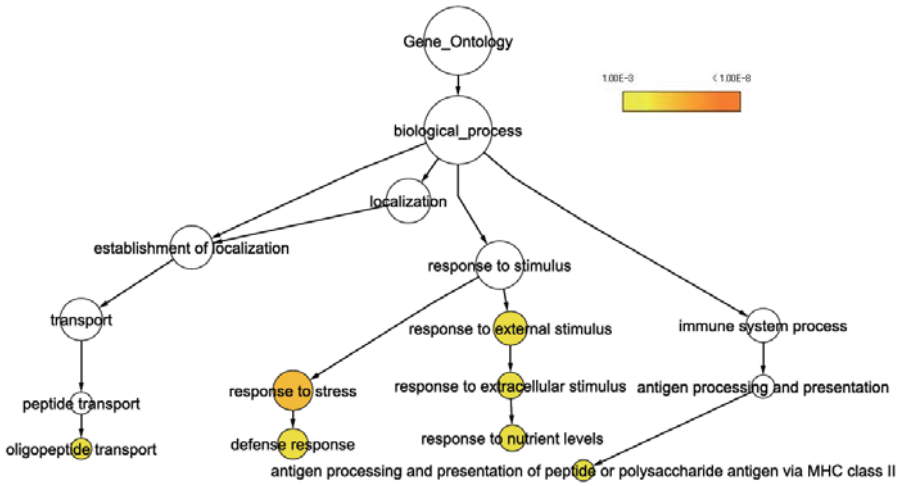

Fig. 1 Significant gene ontology categories (P < 0.001) were extracted from 237 genes showing increased expression.

A-IV (Apoa5), Chemokine (C-X-C motif) receptor 4 (Cxcr4), Apolipoprotein H (Apoh), Membrane-spanning 4-domains, subfamily A, member 1 (Ms4a1), Dipeptidyl-peptidase 4 (Dpp4, Cd26), Protein tyrosine phosphatase (Ptprc, Cd45), T cell receptor beta locus (Tcrb), and Apoptotic peptidase activating factor 1 (Apaf1).

Among cholesterol- and ketone-body-metabolism genes, the expression of 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Hmgcs2) has increased. Among transport genes, the genes with increased expression are Solute carrier family 15 (oligopeptide transporter) and member 1 (Slc5a1) (Table 1).

3.2.2 Genes with Decreased Expression

Genes with decreased expression included urea cycle and lipid metabolic process. Among urea cycle related genes, the genes with decreased expression are Arginase, type II (Arg2), Ornithine carbamoyltransferase (Otc), and carbamoyl-phosphate synthase 1 (Cps1). Among the genes relating to lipid transport and metabolism, the genes with decreased expression are peroxiredoxin 6 (Prdx6) and Peroxisome proliferator-activated receptor gamma (Ppar γ) (Fig. 2 and Table 2).

Table 1 Genes showing increased expression (P < 0.001, Gene ontology categories extracted using BiNGO).

【response to stress】		
Gene name	Definition	UniGene ID
Tnf	tumor necrosis factor	Rn.2275
Aldob	aldolase B	Rn.98207
Clu	clusterin	Rn.1780
Atp6v1g2	ATPase	Rn.158487
Abhd2	abhydrolase domain containing 2	Rn.136611
Sfn	stratifin	Rn.145079
RT1-Ba	RT1 class II, locus Ba	Rn.25717
Ccl5	chemokine (C-C motif) ligand 5,	Rn.8019
Hla-dmb	major histocompatibility complex	Rn.5892
Ada	adenosine deaminase	Rn.12689
RT1-Aw2	RT1 class Ib, locus Aw2	Rn.40130
Apoa4	apolipoprotein A-IV	Rn.15739
RatNP-3b	rat neutrophil peptide-1	Rn.114810
Alb	albumin	Rn.202968
Cxcr4	chemokine (C-X-C motif) receptor 4	Rn.44431
Gsn	gelsolin	Rn.103770
Apoh	apolipoprotein H (beta-2-glycoprotein I)	Rn.1824
Ms4a1	membrane-spanning 4-domains	Rn.16385
Creb3l3	cAMP responsive element binding protein 3-like 3	Rn.20059
Cfd	complement factor D (adipsin)	Rn.16172
Dpp4	dipeptidyl-peptidase 4 (CD26)	Rn.91364
Car3	carbonic anhydrase 3	Rn.1647
Ptprc	protein tyrosine phosphatase	Rn.90166
Bmp2	bone morphogenetic protein 2	Rn.90931
Si	sucrase-isomaltase	Rn.10057
Ephx2	Epoxide hydrolase2	Rn.54495
Tcrb	T cell receptor beta locus	Rn.34871
Adipoq	adiponectin, C1Q and collagen domain containing	Rn.24299
Defa-rs1	defensin alpha-related sequence 1	Rn.122020
Cyp4f5	cytochrome P450 4F5	Rn.10171
Abcc2	ATP-binding cassette	Rn.10265
Apaf1	apoptotic peptidase activating factor 1	Rn.64522
Prnp	prion protein	Rn.3936
Ta4sf4	transmembrane 4 L six family member 4	Rn.13425
【response to external stimulus】		
Gene name	Definition	UniGene ID
Suox	sulfite oxidase	Rn.25720
Bmp2	bone morphogenetic protein 2	Rn.90931
Tnf	tumor necrosis factor	Rn.2275
Si	sucrase-isomaltase	Rn.10057
Clu	clusterin	Rn.1780
Aldob	aldolase B	Rn.98207
Ephx2	Epoxide hydrolase2	Rn.54495
Abhd2	abhydrolase domain containing 2	Rn.136611
Ccl5	chemokine (C-C motif) ligand 5,	Rn.8019
Adipoq	adiponectin, C1Q and collagen domain containing	Rn.24299
Ada	adenosine deaminase	Rn.12689
Apoa4	apolipoprotein A-IV	Rn.15739
Coro1a	coronin	Rn.6990
Apoa1	apolipoprotein A-I	Rn.10308
Cyp4f5	cytochrome P450 4F5	Rn.10171
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Rn.29594
Gsn	gelsolin	Rn.103770
Alb	albumin	Rn.202968
Ms4a1	membrane-spanning 4-domains	Rn.16385
Apoh	apolipoprotein H (beta-2-glycoprotein I)	Rn.1824
Cfd	complement factor D (adipsin)	Rn.16172
Tm4sf4	transmembrane 4 L six family member 4	Rn.13425
Smpd2	sphingomyelin phosphodiesterase 2	Rn.16572

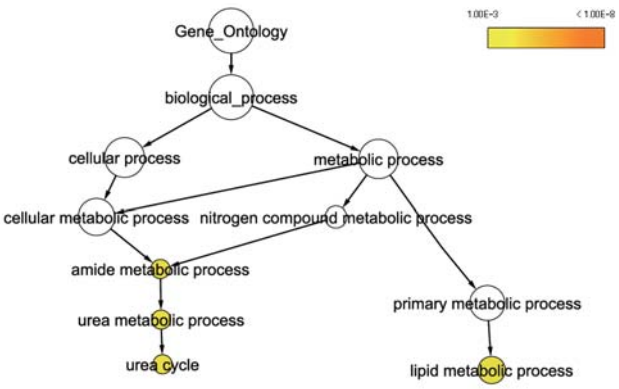

Fig. 2 Significant gene ontology categories (P < 0.001) were extracted from 113 genes showing decreased expression.

【defense response】		
Gene name	Definition	UniGene ID
Ptprc	protein tyrosine phosphatase	Rn.90166
Bmp2	bone morphogenetic protein 2	Rn.90931
Tnf	tumor necrosis factor	Rn.2275
Ephx2	Epoxide hydrolase2	Rn.54495
Tcrb	T cell receptor beta locus	Rn.34871
RT1-Ba	RT1 class II, locus Ba	Rn.25717
Ccl5	chemokine (C-C motif) ligand 5,	Rn.8019
Hla-dmb	major histocompatibility complex	Rn.5892
Defa-rs1	defensin alpha-related sequence 1	Rn.122020
Ratnp-3b	rat neutrophil peptide-1	Rn.114810
Apoa4	apolipoprotein A-IV	Rn.15739
Cyp4f5	cytochrome P450 4F5	Rn.10171
Ms4a1	membrane-spanning 4-domains	Rn.16385
Apaf1	apoptotic peptidase activating factor 1	Rn.64522
Cfd	complement factor D (adipsin)	Rn.16172
【response to nutrient levels】		
Gene name	Definition	UniGene ID
Apoa4	apolipoprotein A-IV	Rn.15739
Suox	sulfite oxidase	Rn.25720
Bmp2	bone morphogenetic protein 2	Rn.90931
Apoa1	apolipoprotein A-I	Rn.10308
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Rn.29594
Gsn	gelsolin	Rn.103770
Alb	albumin	Rn.202968
Si	sucrase-isomaltase	Rn.10057
Aldob	aldolase B	Rn.98207
Adipoq	adiponectin, C1Q and collagen domain containing	Rn.24299
Ada	adenosine deaminase	Rn.12689
【oligopeptide transport】		
Gene name	Definition	UniGene ID
Slc15a1	solute carrier family 15 (oligopeptide transporter)	Rn.10500
RT1-Ba	RT1 class II, locus Ba	Rn.25717
Hla-dmb	major histocompatibility complex	Rn.5892
【response to extracellular stimulus】		
Gene name	Definition	UniGene ID
Apoa4	apolipoprotein A-IV	Rn.15739
Suox	sulfite oxidase	Rn.25720
Bmp2	bone morphogenetic protein 2	Rn.90931
Apoa1	apolipoprotein A-I	Rn.10308
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Rn.29594
Gsn	gelsolin	Rn.103770
Alb	albumin	Rn.202968
Si	sucrase-isomaltase	Rn.10057
Aldob	aldolase B	Rn.98207
Adipoq	adiponectin, C1Q and collagen domain containing	Rn.24299
Ada	adenosine deaminase	Rn.12689
【antigen processing and presentation of peptide or polysaccharide antigen via MHC class II】		
Gene name	Definition	UniGene ID
Ctse	cathepsin E	Rn.92738
RT1-Ba	RT1 class II, locus Ba	Rn.25717
Hla-dmb	major histocompatibility complex	Rn.5892

Table 2 Genes showing decreased expression (P < 0.001, Gene ontology categories extracted using BiNGO).

【urea cycle】		
Gene name	Definition	UniGene ID
Arg2	arginase	Rn.11055
Otc	ornithine carbamoyltransferase	Rn.2391
Cps1	carbamoyl-phosphate synthase 1	Rn.53968
【urea metabolic process】		
Gene name	Definition	UniGene ID
Arg2	arginase	Rn.11055
Otc	ornithine carbamoyltransferase	Rn.2391
Cps1	carbamoyl-phosphate synthase 1	Rn.53968
【amide metabolic process】		
Gene name	Definition	UniGene ID
Arg2	arginase	Rn.11055
Otc	ornithine carbamoyltransferase	Rn.2391
Cps1	carbamoyl-phosphate synthase 1	Rn.53968
【lipid metabolic process】		
Gene name	Definition	UniGene ID
Phlpb	phospholipase B	Rn.91079
Cubn	cubilin (intrinsic factor-cobalamin receptor)	Rn.3236
Hsd3b6	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta	Rn.109394
Prdx6	peroxiredoxin 6	Rn.42
Pparg	peroxisome proliferator-activated receptor gamma	Rn.23443
Hsd11b2	hydroxysteroid (11-beta) dehydrogenase 2	Rn.10186
Aldh1a7	aldehyde dehydrogenase family 1, subfamily A7	Rn.74044
Srd5a1	steroid-5-alpha-reductase	Rn.4620
Comt	catechol-O-methyltransferase	Rn.220
Pcca	propionyl Coenzyme A carboxylase	Rn.6033
Pck1	phosphoenolpyruvate carboxykinase 1	Rn.104376

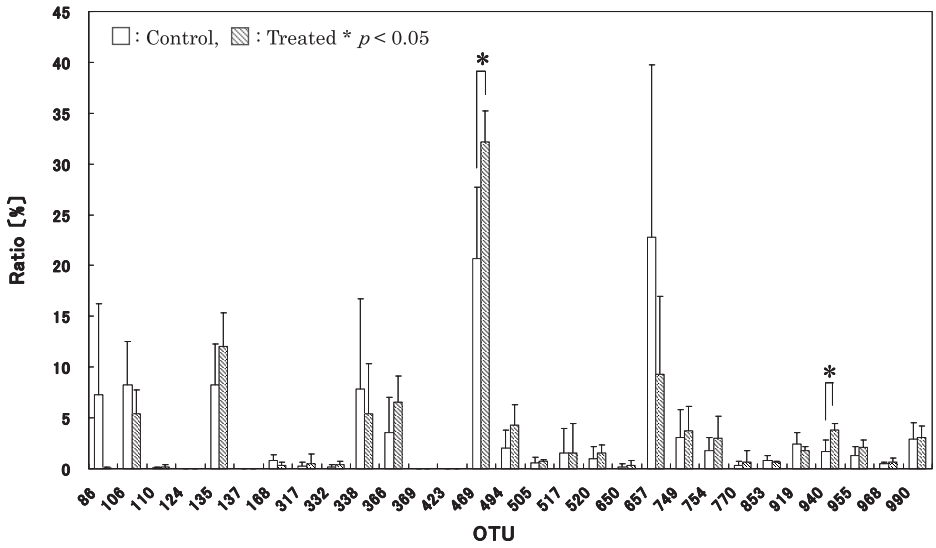

Fig. 3 Fecal specimens were analyzed by T-RFLP analysis (Nagashima method) and presented as the intestinal bacterial flora composition by OTU.

3.3 Intestinal Flora Analysis

The gene expression analysis showed that many immune-related genes were up-regulated in the ileal epithelium of animals administered the *Salacia* extract. Therefore, we have also analyzed the intestinal flora, which is considered to have effect on expression of immune-related genes in intestines.

For the analysis, we used the feces taken from the lower part of the large intestine during the dissection. As the large intestine contains many bacteria difficult to culture, we used T-RFLP analysis to measure the composition ratio of the intestinal flora. T-RFLP analysis profiles intestinal flora accurately without culturing. Based on the composition ratio, we performed clustering using R and drew a phylogenetic tree to measure the similarity in flora pattern. The profiles of the intestinal flora of the test group were different from those of the control group (Fig. 3).

Compared with the Salacia extract-treated group, the intestinal flora of the control group varies largely from rat to rat. They are also distant from one another in the phylogenetic tree, which show little similarity. The profiles of the test group are all similar. All the rats are very close to one another in the phylogenetic tree. These indicate that intake of plant in the genus Salacia results in similar intestinal flora (Fig. 4).

When categorized by phylum, a significant decrease in the ratio of Firmicutes (OTU: 106, 110, 168, 332, 338, 369, 423, 494, 505, 517, 520, 650, 657, 749, 754, 919, 940, 955, 990) was observed, with a significant increase in the ratio of Bacteroidetes (OTU: 366, 469, 853) in the Salacia plant extract-treated group (Fig. 5). That indicates the administration of a Salacia extract has made changes not only in the expression of immune-related genes but in the intestinal flora.

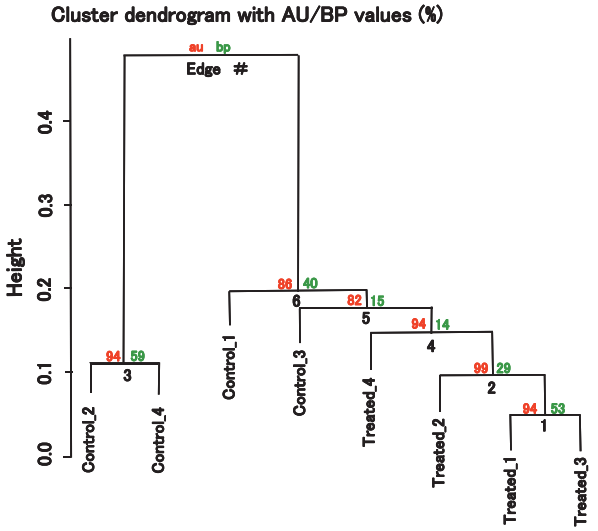

Fig. 4 Cluster analysis was performed on the intestinal bacterial flora composition data determined by T-RFLP analysis (Nagashima method) to construct a phylogenetic tree (au=Approximately Unbiased, bp=Bootstrap Probability).

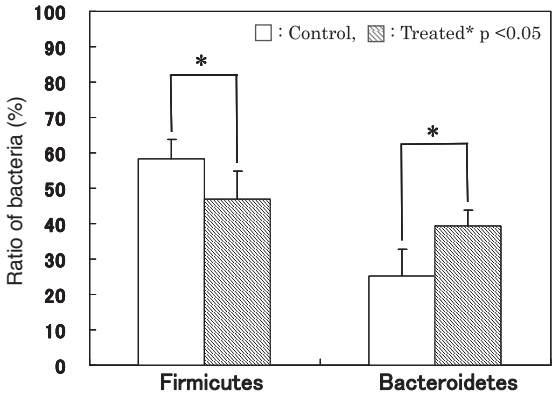

Fig. 5 Proportions of Bacteroidetes and Firmicutes relative to the entire intestinal flora population in the fecal extract administration.

4. Discussion

It has been known that plant in the genus Salacia has many useful effects. This research has been conducted to find out its physiology in the small intestine. As a result, we have found that Salacia makes changes in the expression of many genes in the ileum epithelium. That shows that the genus Salacia has many effects on the intestinal tract. Above all, the changes in the expression of immune-related genes have been found for the first time by this research and the effect is significant. Accordingly, the following discussion will focus on the immune function of Salacia.

Examining the genes with increased expression closely, we have found that they include many genes relating to foreign body recognition, immune system and host defense, especially those relating to Th1 cells. More specifically, they are Ptprc (Cd45)[16] considered to inhibit production of IgE that causes allergies, Th1 related gene Cd26 (Dpp4)[17] contributing to cell immunity, IgG2a[18], which suppresses the invasion of pathogens including various bacteria and viruses, e.g., the influenza virus, and exerts an allergy-suppressive effect, and MHC class II-related genes. Based on the genes identified as showing elevated expression, a possible mechanism of action is proposed, which may operate in the vicinity of Th1 cells (Fig. 6)[19].

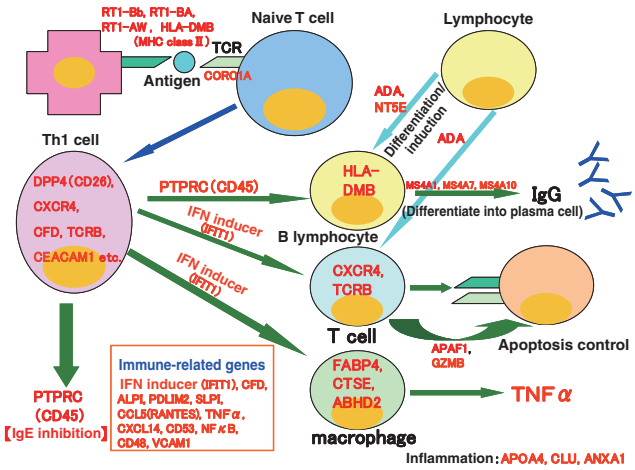

Fig. 6 Possible mechanism of action speculated from the genes identified as showing increased expression in the vicinity of the Th1 cells (genes listed in Table 1 are shown in red, genes identified among the 237 genes showing increased expression but not used in the functional categorization by BiNGO are presented in black).

In our previous study, we found that Salacia extracts had the effect of decreasing putrefied products and ammonia in intestines. We infer that the decrease of ammonia in the intestines by a Salacia extract has resulted in a decrease in the expression of urea cycle related genes (Cps1, Arg2 and Otc) in the epithelium of small intestine[20].

The types of bacteria and their composition in intestinal flora are closely connected with intestinal immunity. In our analysis of the intestinal flora, the flora patterns that varied from rat to rat have become similar to one another due to the administration of a Salacia extract. The percentage of Bacteroides has increased. The immunostimulatory function of this phylum of bacteria is attracting attention. They exhibit a stronger immune function than lactic acid bacteria well known for their immune activation. It has been found that Bacteroides increase production of a cytokine relating to IgA and biodefense[21-22]. In our experiment, two OTUs of Bacteroides (366 and 469) drastically increased in percentage. We studied the homology using the base sequences obtained by cloning. The result shows that it is highly possible that the two OTUs include Bacteroides acidofaciens, one of the Bacteroides having particularly high immune activation effect. Also, some research shows LPS existing in the cell walls of Bacteroides has the immune activation effect[23].

All these indicate that a Salacia extract has effects on intestinal flora and that the changed flora acts on the immune system of the lower part of the small intestine. In this research, expression of many transport and metabolism related genes has also changed. These genes work closely with the liver. We would like to continue study on these genes.

The result of this research may not necessarily hold true for humans. We have conducted the experiment on the rats in clean environment and under strictly managed conditions, such as the temperature and feed. There are no bifidobacteria, which exist in large quantity in human intestines[24]. However, we have confirmed that a Salacia extract does make changes in human intestinal flora. We believe there is great possibility it acts on human immune functions. We would like to verify its functions on humans.

Although plant in the genus Salacia has been used in Ayurveda for many years, not much about its functions is known. However, the biological regulation through intestinal immunity is connected with many diseases that are said to be improved by Salacia extracts. We are convinced that this research has revealed some of the functions of Salacia extracts.

We launched functional food containing a Salacia extract, Metabarrier (Fig. 7), in 2007. Through research of functionality of food ingredients to develop products using still more highly functional ingredients, we will continue our efforts to help enhance people's quality of life.

Fig. 7 Metabarrier.

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