REVIEW

The intestinal microbiota and its role in human health and disease

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Abstract : The role of the intestinal microbiota in human health is gaining more attention since clear changes in the composition of the intestinal bacteria or environment are seen in patients with inflammatory bowel disease, allergy, autoimmune disease, and some lifestyle-related illnesses. A healthy gut environment is regulated by the exquisite balance of intestinal microbiota, metabolites, and the host's immune system. Imbalance of these factors in genetically susceptible persons may promote a disease state. Manipulation of the intestinal microbiota with prebiotics, which can selectively stimulate growth of beneficial bacteria, might help to maintain a healthy intestinal environment or improve diseased one. In this review, analytical methods for identification of intestinal bacteria and an update on the correlation of the intestinal microbiota with human health and disease were discussed by introducing our recent studies to determine the prebiotic effects of a fiber-rich food in animal model and on healthy people and patients with ulcerative colitis (UC). J. Med. Invest. 63 : 27-37, February, 2016

Keywords **:** *intestinal microbiota, symbiotic relationship, ulcerative colitis, Bacteroides, organic acid*

1. GENERAL FEATURE OF HUMAN INTESTINAL MICROBIOTA

Human are borne in a practically germ-free state. Shortly after, various microorganisms derived from the mother or environment start to settle in many places of the body. Bacterial colonization of the intestine is essential for its proper development and health. The relationship between intestinal bacteria and health was realized with the establishment of anaerobic culture methods and germ-free breeding systems. Studies with axenic animals demonstrate that colonizing microorganisms stimulate the maturation of intestinal tissue, host metabolism, and absorption of nutrients, and they fortify host protection systems such as mucus production and secretory IgA (1). The effects of colonization are particularly strong in the intestinal tract, where many kinds of bacteria live and the total amount outnumbers host cells. A recent review by Rajilić-Stojanović and de Vos (2) compiled the characteristics of more than 1000 species of microorganisms common to the human intestine. *Escherichia coli* and *Enterococcus* are first detected just after birth ; exposure probably occurs in the birth canal and the immediate environment. During nursing, *Bifidobacterium* and *Lactobacillus* become dominant. After weaning, various kinds of bacteria have colonized the intestine and volume and diversity of microbiota increased to the same level as in an adult. The relationship between host and microbiota is symbiotic ; the host's intestine provides a place for bacteria to grow and supplies nutrition in the form of undigested food and removed mucus, while the bacteria ensure proper tissue and immune development of the intestine as mentioned above. These resident bacteria form a complex network that utilizes various energy sources from the host or other bacteria to survive in the intestine. Growth, however, does not go unchecked. The composition of intestinal microbiota is affected by many factors including the host immune system, genetics, and environmental elements. Thus, the proper functioning of host systems and the microbiota is vital to intestinal homeostasis.

Comparing the differences in intestinal microbiota between healthy and disease states or when diet, lifestyle, or disease condition are altered clarifies the roles of intestinal bacteria in human health. The intestinal bacterial population is complex, and identification of some species is difficult since they cannot be cultured. Recent advances in sequencing techniques have improved the ability to analyze the microbiota composition.

Received for publication June 8, 2015 ; accepted November 4, 2015.

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2. ANALYTICAL METHODS USED TO STUDY THE INTESTINAL MICROBIOTA

In combination with germfree breeding system, development of anaerobic culture technique have exceedingly contributed to clarify the roles of intestinal bacteria in human health. Constantly advancing technique for DNA sequencing has made analysis of intestinal microbiota more easily within shorter period. Now we will be able to study intensively about how difference of bacterial composition influence the pathogenesis of life-style related pluricausal diseases, why a population of bacteria increased under the pathogenic state, what kind of intervention will improve the patient's condition. Characteristics of analytical methods used for intestinal bacteria are described below. Combination of adequate method is important for deeply understanding the role of intestinal bacteria.

Culture method

In the method of Mitsuoka *et al.* (3), diluted fecal samples were spread onto nonselective (e.g. BL agar, EG agar) and selective media (e.g. Rogosa SL agar, DHL agar), and then were cultured under aerobic or anaerobic conditions. Physical features of the colonies, Gram staining, and biochemical properties of the isolates were used to determine their genus or species. The viable number of identified bacteria were then calculated. Culture methods for analysis of intestinal bacteria are difficult for several reasons. For one, an expert is usually required to discriminate between colonies.

In addition, growth of bacteria is influenced by many factors including the constitution of the media or the manner in which the cultures were pretreated. Despite the difficulties, a small number of bacteria in the intestine can be discovered with the appropriate selective media. Isolated bacteria are then placed in a gnotobiotic animal model to investigate its functions. For strict anaerobes that are very difficult to culture (e.g. extremely-oxygen sensitive *Clostridium*), a whole fecal sample treated with chloroform was inoculated into a germ-free animal intestine as described by Momose *et al.* (4)

Culture-independent methods

Almost culture-independent methods target 16S ribosomal RNA gene (16S rDNA), which is common among prokaryote and consisted from highly conserved and variable regions. Conserved regions are utilized as primer binding site in amplification or sequencing of this gene, and difference of DNA sequence in variable regions are important for discrimination of genus or species of bacteria. Recently advancing metagenomic analysis targets 16S rDNA or whole genome in the samples. Anyway, complete extraction of bacterial DNA from samples is important for precise analysis. Physical destruction with glass beads, enzymatic hydrolysis (5) or benzyl chloride (6) were used to destroy the bacterial cell wall. We extracted DNA from fecal samples by the method of Morita *et al.* (5). Briefly, fecal samples were washed with a solution of 10 mM Tris-HCl and 50 mM EDTA (pH 8.0) to remove PCR inhibitors such as bile acid and some food-derived components, and were treated with achromopeptidase, lysozyme and proteinase, and finally with SDS for complete lysis. Complete lysis of bacteria was confirmed by Gram staining.

Among 16S rDNA-targeting methods, terminal-restriction fragment length polymorphism (T-RFLP) analysis is good at finding time-dependent changes or the effect of some food components on an individual's microbiota. In our previous studies, intestinal microbiota was analyzed according to the method of Sakamoto *et al.* (7). The procedure is outlined in Figure 1 and used primers are shown in Table 1. The bacterial 16S rDNA was amplified by PCR using 5'-6-carboxyfluorescein (6-FAM) -labeled 27F primer and 1492R primer except for *Bifidobacterium* (7). Then, PCR products were digested with restriction enzymes *Hha*I and *Msp*I, and 5'-labeled restriction fragments were examined in a genetic analyzer. Polymorphisms of the fragments reflect the constituents of intestinal microbiota. The sizes of 5'-labeled restriction fragments vary due to the genus- or species-dependent difference of digestion patterns, so the unique size of the fragments aid in identification. Although the exact species of changing bacteria cannot be determined, it can be supposed from the size of the T-RF peak by computer simulation with a phylogenetic assignment database for T-RFLP analysis of human colonic microbiota as described by Jin *et al.* (8) and Matsumoto *et al.* (9), or by Microbiota Profiler software (Infocom, Tokyo, Japan). By clustering the T-RFLP profiles and combining them with individual information such as dietary habits and disease states, we can consider which bacterial change has an important effect on healthy or disease conditions. Because of easy operation and comparative inexpensiveness, T-RFLP is suitable for screening out changes or differences of microbiota composition. But PCR bias is the inevitable problem due to fidelity of primers or efficiency of amplification. Nagashima *et al.* (10) proposed new primer-enzyme combinations to T-RFLP profiling of bacterial populations. They amplified 16S rDNA with using fluorescently labeled 516f primer and 1510r primer and then digested with *Rsa*I plus *Bfa*I or with *Bsl*I. They mentioned that this new protocol made it easy to predict what kind of intestinal bacterial group corresponded to each T-RF including *Bifidobacterium.*

To determine the amount of targeted bacteria correctly, genus- or species-specific real-time PCR is a powerful method. We confirmed the results of T-RFLP by genus-specific real-time PCR with primers in Table 1. By using cloned 16S rDNA prepared from standard strains, we determined the copy number of the 16S rRNA gene

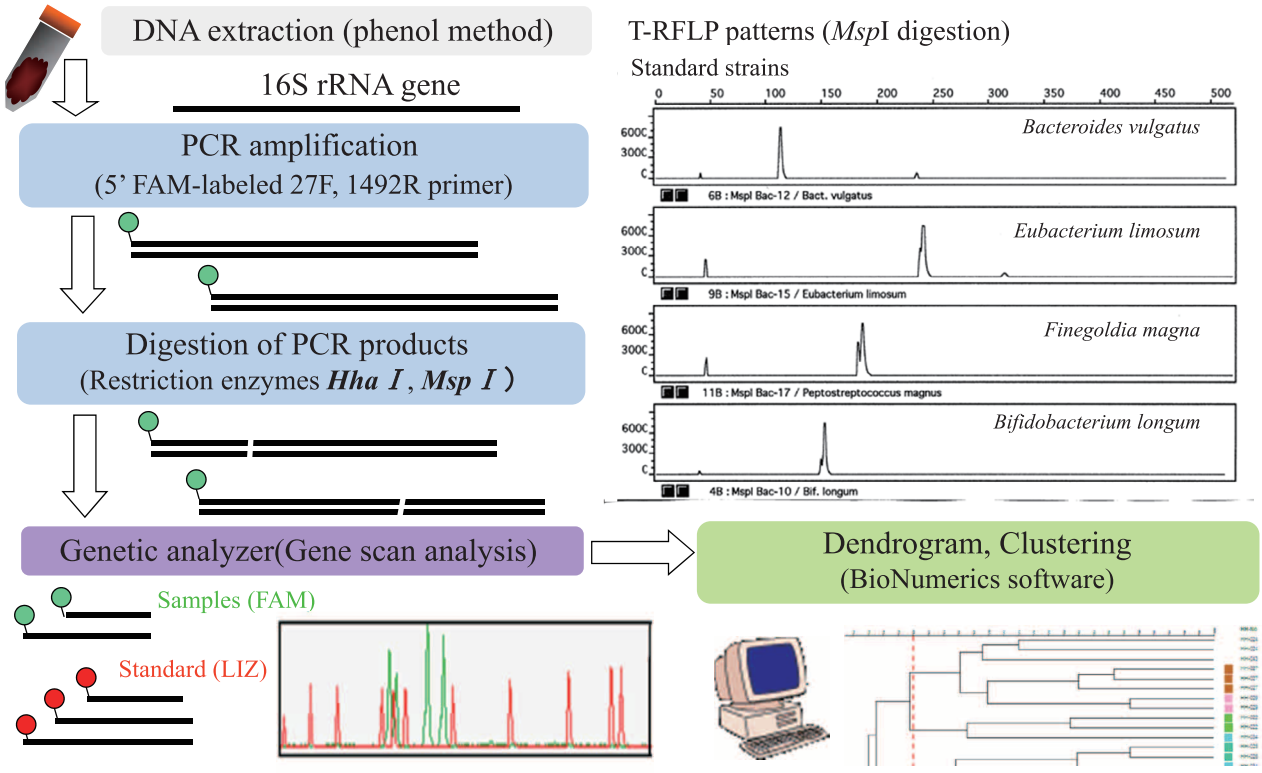

Figure 1. Outline for analysis of intestinal microbiota by Terminal-RFLP.

Table 1 Primers used for T-RFLP and quantitative PCR

Assay/object	Primer	Sequence	Reference
T-RFLP	27F-FAM	6FAM-AGAGTTTGATCCTGGCTCAG	7
	1492R	GGTTACCTTGTTACGACTT	
Bifidobacterium	Bif164F	CATCCGGCATTACCACCC	11
	Bif662R	CCACCGTTACACCGGGAA	
Sulfate-reducing bacteria	Des-f	CCGTAGATATCTGGAGGAACATCAG	12
	Des-r	ACATCTAGCATCCATCGTTTACAGC	
Enterococcus	Enc-F	CCCTTATTGTTAGTTGCCATCATT	13
	Enc-R	ACTCGTTGTACTTCCCATTGT	
Clostridium subcluster I	CI-F1	TACCHRAGGAGGAAGCCAC	14
	CI-R2	GTTCTTCCTAATCTCTACGCAT	
Clostridium subcluster XI	CXI-F1	ACGCTACTTGAGGAGGA	
	CXI-R2	GAGCCGTAGCCTTTCACT	
Clostridium subcluster XIVab	CXIV-F1	GAWGAAGTATYTCGGTATGT	
	CXIV-R2	CTACGCWCCCTTTACAC	
Clostridium common probe	Clostridium probe	6FAM-GTGCCAGCAGCCGCGGTAATACG-TAMRA	
Bacteroides	AllBac296F	GAGAGGAAGGTCCCCAC	15
	AllBac412R	CGCTACTTGGCTGGTTCAG	
	AllBac375Probe	6FAM-CCATTGACCAATATTCCTCACTGCTGCCT-TAMRA	

from several bacteria (16, 17). Recently, a new quantification system based on reverse transcription-quantitative PCR targeting of multicopy rRNA molecules was established by Matsuda *et al.* (18). They demonstrated the precise detection of subdominant populations such as *Staphylococcus* and *Pseudomonas* or those only detected at lower incidences by quantitative PCR and culture methods.

16S rDNA-targeting clone library method was used for phylogenetic analysis of complex microbiota including unknown bacteria (19, 20). 16S rDNA was amplified by PCR with lower cycles to reproduce the original composition of microbiota, and PCR products were cloned and used for sequencing. Clustering of the 16S sequences into phylum group and the frequency of the clones in each phylum group entirely described the composition of microbiota. Representative 16S sequence in each group is used for homology search to identify the bacteria. More complicated steps, longer time and more cost were needed compared to T-RFLP or other methods. However, recent advances in sequencing technology have resolved this problem.

Next generation sequencer can do large-scale sequencing accurately and reproducibly. It contributes to rapid analysis of complex intestinal microbiota. As summarized by Yamada (21), 16S rDNA-targeting metagenome analysis is very helpful to know the difference of complicated microbiota phylogenetically. In a general metagenomics analysis, DNA fragments from whole genome are directly sequenced. Enormous sequence data is assembled into contig, and compared with sequences in common databases to assign their functions. By comparing the composition of functional genes between healthy and diseased state, we can know which function of intestinal microbiota have a key role in maintenance of health or in the pathogenesis of many kinds of disease, although a high degree of data processing technique with more time and cost are needed for this method.

Fluorescence *in situ* hybridization (FISH) can visualize the behavior of intestinal bacteria in the intestinal environment with keeping the tissue structure. Since intestinal bacteria contain fairly large amount of rRNA, genus- or population-specific probes for 16S rRNA have been developed to examine their spatial distribution (22). If we will use multiple probe labeled with different fluorescent dyes, we can find an abnormality of bacteria's location and changes of bacterial composition. Intestinal bacteria was perfectly separated from epithelium by secreted mucus in the healthy condition, but penetration of bacteria into the mucus layer have been observed in experimental models and in patients with inflammatory bowel diseases (23, 24).

Analysis of metabolites produced by intestinal microbiota

Intestinal microbiota affects human health directly or indirectly through their metabolites. Bacteria present in the lower intestinal tract metabolize indigestible dietary fiber and mucus components into short chain fatty acids (SCFA) such as propionic acid and butyric acid. These organic acids are absorbed into the colonic epithelium where they are used for many purposes, including : being used as an energy source, stimulating growth, differentiation, and mucin production of epithelial cells, fortifying intestinal barrier functions, and regulating the immune response (25, 26). SCFA also functions as modulator in fatty acid metabolism by stimulating secretion of GLP-1 and peptide YY, and in energy expenditure through enteric nervous system (27). The other absorbable metabolites have been associated with systemic diseases. Trimethylamine (TMA) produced by intestinal bacteria from dietary choline and L-carnitine, was further metabolized to trimethylamine-*N*-oxide (TMAO) and promoted atherosclerosis (28). Hsiao *et al.* (29) demonstrated through serum metabolome analysis that 4-ethylphenylsulfate, a uremic toxin produced by intestinal bacteria, was absorbed under dysbiotic condition and involved in the autism.

3. EFFECTS OF A FIBER-RICH FERMENTED FOOD ON THE RAT INTESTINAL MICROBIOTA

We analyzed the effect of fermented brown rice (FBRA), which is rich in dietary fiber, on the rat intestinal microbiota. Among cultured bacteria, *Lactobacillus* significantly increased in the 10% FBRA-fed group compared to the basal diet-fed group. T-RFLP analysis also showed that FBRA substantially affected the intestinal microbiota (30). The T-RF peak corresponding to the *Lactobacillus* genus significantly increased with 10% FBRA feeding, and the fragment size of the T-RF peaks suggested an increase of *Lactobacillus*

acidophilus, L. johnsonii, and *L. intestinalis* species (Figure 2a). T-RFLP profiles of fecal microbiota in the 10% FBRA-fed group showed a different cluster from the basal diet-fed group (Figure 2b). Some lactic acid bacteria were isolated directly from the FBRA itself, but they were not the same species as those isolated from rat intestine as shown by randomly amplified polymorphic DNA (RAPD) analysis. These results indicate that addition of FBRA to the rat diet increases the resident *Lactobacillus* in the intestine.

Dextran sulfate sodium (DSS) treatment of rats induces colitis with similar histological features to human ulcerative colitis (31). We showed that addition of FBRA to the diet had an inhibitory effect on formation of ulcers in the rat DSS-induced colitis model. Myeloperoxidase activity in colonic mucosa, a marker of neutrophil infiltration, also significantly decreased in the 10% FBRA-fed rats. DSS treatment caused a decrease in fecal *Lactobacillus*, but the decrease was suppressed in FBRA-fed rats (32).

How *Lactobacillus* correlates with the suppression of colitis was not further studied in this work, but some strains of *Lactobacillus* have been reported to fortify intestinal barrier functions and to regulate excess immune response by inducing regulatory T cells (33, 34). FBRA contains a large amount of two types of dietary fiber, β-glucan and arabinoxylan, which can be used as a carbon source

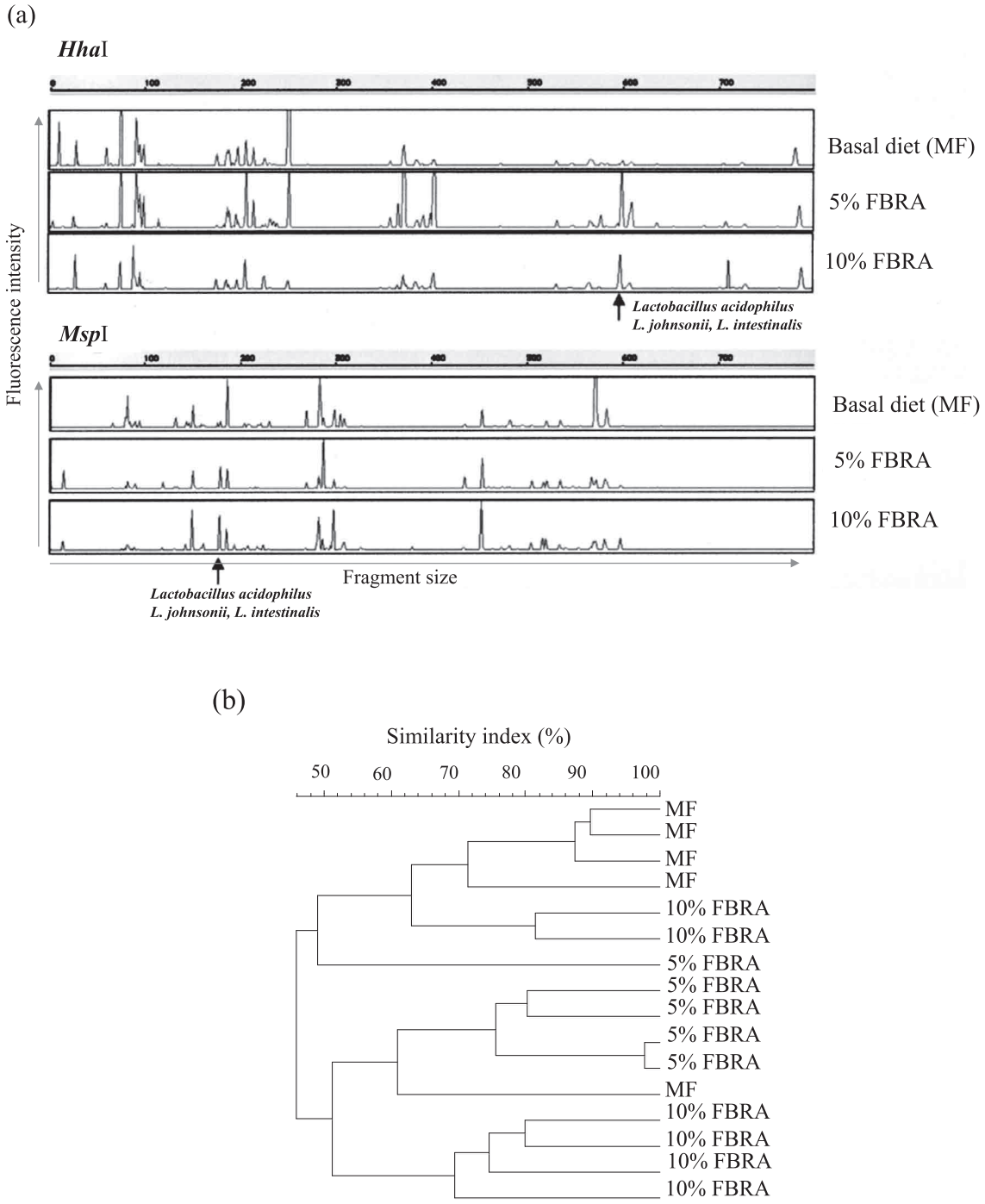

Figure 2. Rat fecal microbiota analyzed by Terminal-RFLP. Representative T-RFLP profiles (a) and dendrogram analysis (b) of rats fed a basal or FBRA-containing diet. (Cited from reference number 30)

by *Lactobacillus* and could help to maintain a beneficial number of the bacteria in DSS-treated rats. Lactic acid produced by *Lactobacillus* is further metabolized to SCFA, such as butyric acid, by metabolic cross-feeding among many kinds of intestinal bacteria. Members of *Negativicutes* such as *Veillonella* and *Megasphaera*, which are gram-negative staining and abundant in gastrointestinal tract, utilize lactic acid and acetic acid to produce SCFA (2). Bacteria-derived organic acids have been shown to induce anti-inflammatory effects and intestinal barrier fortification (26).

4. EFFECTS OF A FIBER-RICH FERMENTED FOOD ON THE HUMAN FECAL MICROBIOTA

Based on the increased number of resident *Lactobacillus* in rats (30) and the suppressive effect in the DSS-induced colitis model (32), we planned to estimate beneficial effects of FBRA in human by randomized placebo-controlled crossover trial. In the first study, effects of FBRA on the fecal microbiota and bowel function in healthy adults were examined (16). While FBRA supplied the Ministry of Health, Labour and Welfare, Japan's recommended daily intake of fiber, no significant effects on the intestinal microbiota composition and organic acids concentration were found after ingestion of FBRA for 2 weeks. This was in contrast to *in vitro* results ; addition of FBRA to fresh fecal samples showed increase of organic acids (SCFAs) and T-RF peaks that corresponded to *Clostridium* subcluster XIVab and *Bifidobacterium*. Probably human adults have very stable intestinal microbiota and is not easily influenced by the dose and ingestion period used in this study.

In another study to estimate the effectiveness of FBRA in UC patients under leadership of Dr. Hideki Ishikawa (Kyoto Prefectural University), we covered to examine the change of fecal microbiota. Subjects ingested 21 g of FBRA or control food per day (the same dose as in the healthy adults) for 3 months. Before and after the first-eating period T-RFLP profile of fecal microbiota and bacterial metabolites showed no significant difference (unpublished data), while fecal microbiota of UC patients before the intervention was different from that of healthy adults as described in section 5.

Many clinical trials for UC patients showed beneficial effects of probiotics (35, 36), and recently VSL#3 has been demonstrated to be effective in maintenance/induction of remission and decrease of clinical disease activity in large-scale, randomized, double-blind, placebo-controlled trial (37). Synbiotics consisted from *Bifidobacterium breve* and galacto-oligosaccharide (38) or *Bifidobacterium longum* and psyllium (39) also ameliorated colitis or patient's quality-of-life. However, only a few clinical trials have suggested beneficial effects of prebiotics in UC patients. Oligofructose-enriched inulin (12 g/day for 2 weeks) was tested in a randomized, placebo-controlled, double-blind pilot trial (35, 40). Intestinal inflammation evaluated with a fecal concentration of calprotectin was reduced significantly at the middle point only in the group receiving oligofructose-enriched inulin. But at the end of the study period, disease activity scores were significantly reduced in both group. Germinated barley foodstuff (GBF) is a dietary component high in glutamine-rich protein and hemi-cellulose-rich dietary fiber, and showed suppressive effect in experimental colitis and patients with UC (35, 41-45). In small-scale multi-center open trial, patients treated with 20-30 g of GBF for 24 week showed significant decrease in clinical activity index (CAI) (41). Randomized controlled trials also showed decrease of CAI and prolonged remission period by 20 g of GBF for 12 months (42), reduced level of serum TNF-α, IL-6, IL-8 and serum CRP level by 30 g of GBF for 2 months (43, 44). However, a large, double-blind, placebo-controlled trial is not yet reported.

Verification of prebiotic potential in human may probably need an adequate dose and ingestion period for individuals. In the above clinical trials with GBF, considerable doses of GBF were used for longer period. Enhanced luminal butyrate production by GBF treatment (45) might improve the patient's symptoms, and high water-holding capacity of GBF itself could contribute the amelioration. If a functional component which can improve intestinal environment was contained, like as GBF-mediated regulation of the fecal water content, effects of prebiotics might appear more clearly. Since human intestinal microbiota has individually different composition even at the start of clinical trials, growth of beneficial bacteria is stimulated by prebiotics to various degrees. Efficiency of metabolic cross-feeding between intestinal bacteria also affect the constitution of SCFA in the intestine. Time-, diet- and disease-dependent change of bacterial composition could possibly interfere the action of prebiotics and concentration of effective metabolites. We compared bacterial composition and SCFA concentrations in feces in healthy adults before and after experimental food, *in vivo* effects were difficult to detect due to the large inter-individual variation. In UC patients, disease state (remission or clinically active) influenced the concentration of SCFA. Reduced diversity of intestinal microbiota in UC patients (described in section 5) may also be related to indistinct results in the trials.

5. COMPARATIVE ANALYSIS OF THE FECAL MICROBIOTA BETWEEN HEALTHY ADULTS AND PATIENTS WITH ULCERATIVE COLITIS

The healthy gut environment is controlled by a complicated balance of the intestinal microbiota, their metabolites, and the host immune system. Imbalance of these factors can promote disease states like inflammatory bowel disease. Since the two clinical studies described above were run at the same time, we compared the microbiota in UC patients before experimental food with those in healthy adults and found a reduced diversity and imbalance of fecal microbiota in UC patients (17). The total number of T-RF peaks was significantly lower in patients in remission than in healthy adults. Clustering analysis of T-RFLP profiles indicated that UC patient's microbiota formed different clusters from those of healthy subjects without dependence on disease activity (Figure 3b). T-RFLP analyses in UC patients showed decreasing peak area which corresponded to *Clostridium* and *Bacteroides* (Figure 3a). Decrease of genus *Bacteroides* and *Clostridium* subcluster XIVab was confirmed by genus- or species-specific real-time PCR (Figure 4a). Probably due to the decrease of these dominant anaerobes, we observed an increase of *Enterococcus* by culture and real-time PCR in UC patients. Medical treatment and intake of probiotics did not affect the differences in the microbiota between healthy and UC subjects.

Bacteroides and *Clostridium* subcluster XIVab belong to the dominant phyla *Bacteroidetes* and *Firmicutes* in the human intestinal microbiota, and they cooperatively ferment undigested food components to SCFAs by metabolic cross-feeding (46-48). Consistent with the reduction of these anaerobes, there was a lower concentration of fecal organic acids in UC patients compared to healthy adults (Figure 4b). These results indicate that dysbiosis occurred in UC patients. Similar features have been reported for intestinal microbiota in UC and Crohn's disease patients (49-51). Andoh *et al.* (49) showed the altered T-RFLP patterns in UC patients. Some T-RFs, derived from the unclassified bacteria, *Ruminococcus, Eubacterium, Fusobacterium,* gammaproteobacteria, unclassified *Bacteroides*, and unclassified *Lactobacillus* were detected in the UC patients, but not in the healthy individuals. Such difference was also found between the active UC patients and remission patients. Nishikawa *et al.* (50) compared the mucosa-associated microbiota between UC patients and non-inflammatory bowel disease (IBD) controls by T-RFLP analysis. They showed that active UC patients

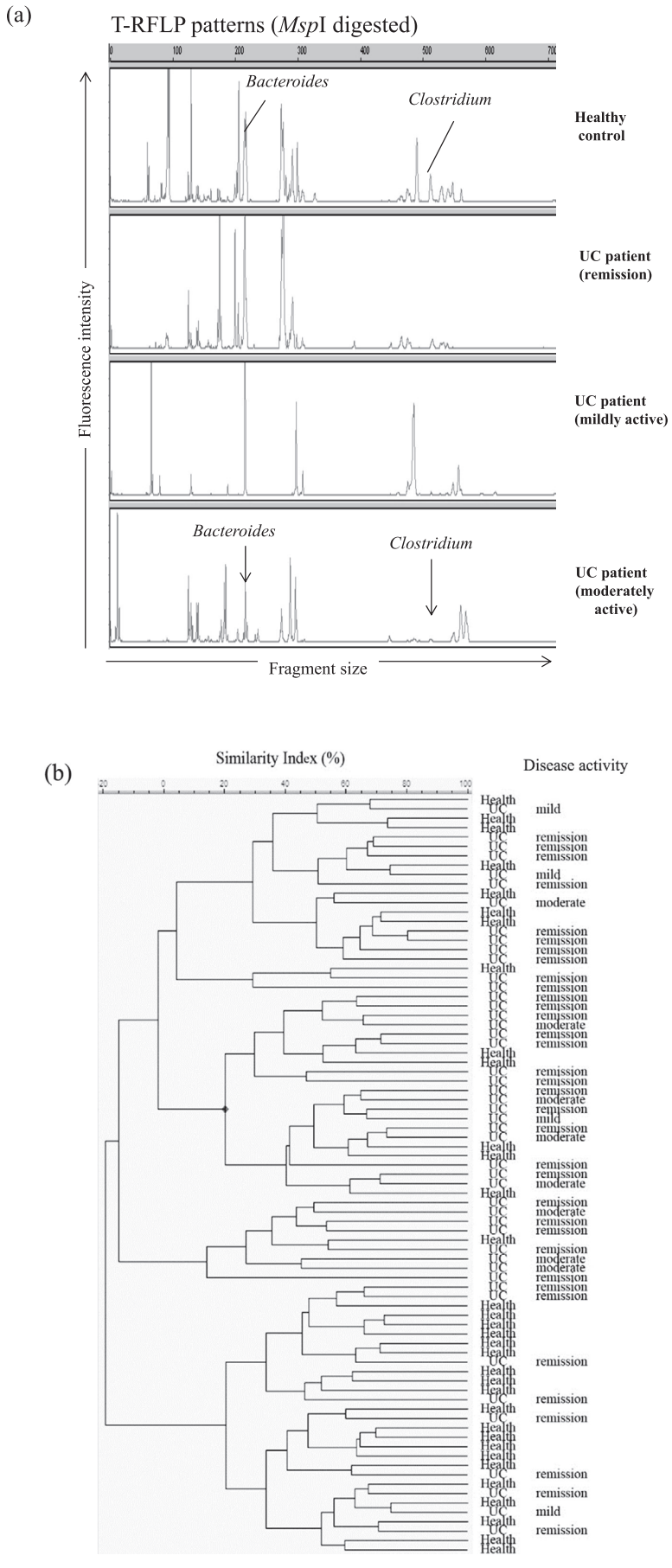

Figure 3. Comparison of intestinal microbiota between healthy adults and patients with ulcerative colitis. Representative T-RFLP profiles (a) and dendrogram analysis (b) of fecal microbiota in healthy control and UC patients. (Cited from reference number 16 and 17, and revised)

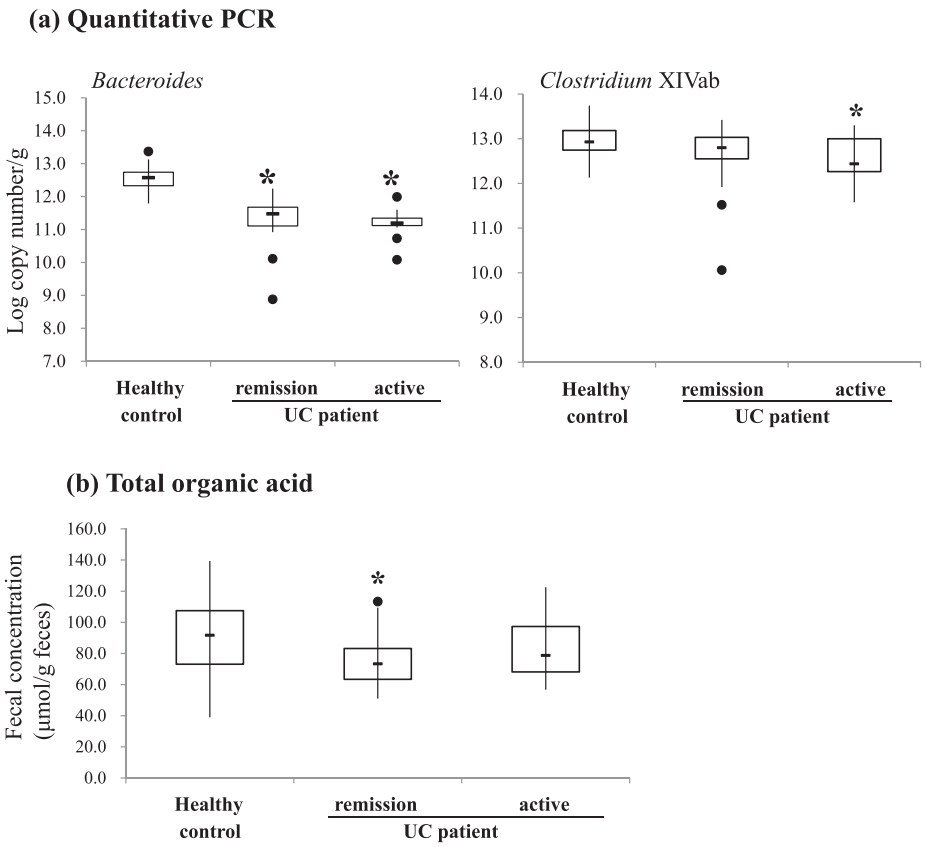

Figure 4. Decrease of dominant anaerobes and their fermentative metabolites in UC patients. Quantitative PCR for *Bacteroides* and *Clostridium* subcluster XIVab (a) and fecal concentration of organic acids (b) are shown. *Significant difference from healthy control ($p < 0.05$, Mann-Whitney *U*-test). (Cited from reference number 16 and 17, and revised)

possessed significantly fewer diverse microbial compositions, but increased diversity of microbiota was observed in remission phase of identical patients. Reduced diversity of faecal microbiota in Crohn's disease (CD) was revealed by Manichanh *et al.* (51). They compared two DNA libraries constructed from faecal samples from heathy donors and patients with CD, and identified 125 non-redundant ribotypes, mainly represented by the phyla *Bacteroidetes* and *Firmicutes*. Number of distinct ribotypes belonging to *Firmicutes* was less in library from CD patients than that from healthy donor. Significant reduction of this phylum was confirmed by fluorescent *in situ* hybridization directly targeting 16S rRNA in faecal samples. In this study, we compared fecal microbiota at only one time point ; however, the intestinal microbiota has been reported to be unstable and easily changed during clinical remission (52). Therefore, further investigation of samples from UC patients at different stages of remission and of samples from different sites are required to confirm the microbiota differences between UC patients and healthy individuals seen in this study.

6. CHANGES OF THE INTESTINAL MICROBIOTA IN CHRONIC INFLAMMATORY BOWEL DISEASES AND LIFESTYLE-RELATED DISEASES

The intestinal tract is an important organ for absorption of nutrients, and it functions as a physical and biochemical barrier against food-derived antigens and pathogenic microorganisms. Development of these protective functions is stimulated by colonization of commensal bacteria. Closely crowded commensal bacteria in the lumen or on the surface of intestinal mucosa competitively prevent growth of pathogenic bacteria of foreign origin (53). Enhanced production of mucin, antibacterial peptides, and secretory IgA associated with commensal bacteria in the intestine has also been reported (1, 25, 53, 54). These resident bacteria also contributed to the enhancing effect through their colonization and their metabolite organic acids (25, 26). Balanced microbiota is necessary for producing organic acids with adequate amount and proportion because of metabolic cross-feeding among anaerobic intestinal bacteria (46-48). Colonization of some commensals, such as *Bacteroides* and *Bifidobacterium*, has been reported to fortify the intestinal barrier by stimulating expression of antibacterial substances and intercellular adhesion molecules, and to suppress inflammation by modulating transduction of inflammatory signals or by inducing regulatory T cells (Treg cells) (34). *Clostridium* has also been demonstrated to suppress an excessive immune response by induction of Treg cells, in part through the fermentation metabolite butyrate (55, 56).

As summarized in Figure 5, colonized bacteria stimulate the maturation of the human intestine and form a symbiotic relationship through interaction with host defense systems. The human host provides a place and nutrients for the microbiota to grow while maintaining an adequate distance from the microbiota through innate immune mechanisms. When this exquisite balance is collapsed, the host immune cells attack resident bacteria ; persons with certain genetic deficiencies in regulation of the immune response may experience chronic intestinal inflammation when this occurs.

Chronic intestinal disease may be caused by a mixture of defects of immune response genes of the host and environmental factors. Pathogenesis of Crohn's disease, a chronic inflammatory bowel

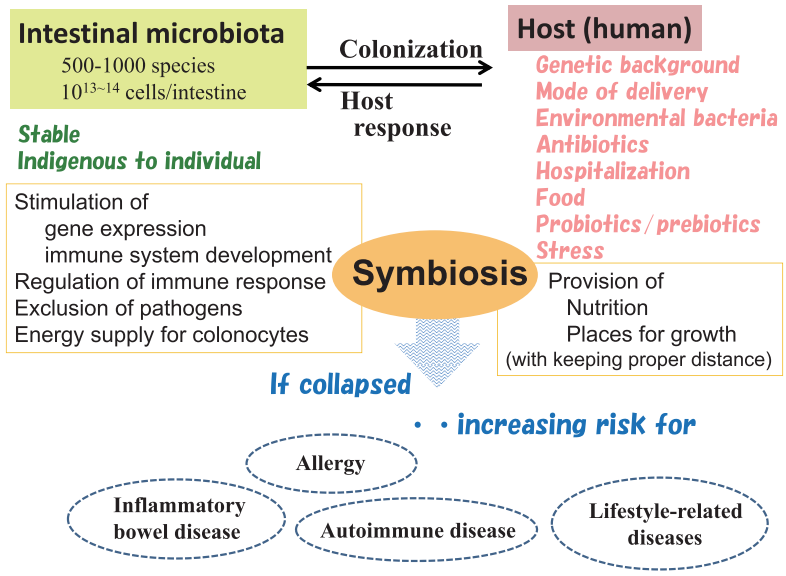

Figure 5. The symbiotic relationship between the host and the intestinal microbiota contribute to human health.

disease (IBD), includes genetic deficiency in pathogen sensor molecules such as NOD2 and TLR5 and autophagy gene (57-59). In ulcerative colitis, the protective mucosal layer of the colon is lost because mucin-producing goblet cells are reduced ; however, the genetic causes have not yet been clarified. NOD2 is a sensor for intracellular microorganisms. TLR5 recognizes flagellin of penetrating bacteria, and it sends signals to induce production of antibacterial peptides and sIgA. Atg16L1 is responsible for the generation of autophagosome, and plays an important role in degradation of intracellular bacteria, and in regulating the secretion of proinflammatory cytokines (58, 60).

Defects in these molecules reduce certain protective functions in the intestine that maintain the distance between host and commensal bacteria, resulting in an immune response to resident bacteria. In addition to genetic vulnerability, environmental factors such as overwork, stress, and infection may trigger these intestinal diseases. These factors cumulatively cause a collapse of the symbiotic relationship, at which point the host immune system attacks the intestinal commensal bacteria. Chronic colitis is thought to be the result of a combination of continuous inflammation against commensal bacteria, lowered regulation of the immune response, and reduced tissue repair activity. Decrease of dominant bacteria and less diversity in intestinal microbiota are now considered common features of IBD. Restoration of balance of the intestinal microbiota may be one of the only effective treatments for patients. Recently, bacterial transplantation has been demonstrated to be effective in patients with UC (61) and in colitis caused by repeated antibiotic treatments for *Clostridium difficile* infection (62).

Changed intestinal microbiota and dysbiosis are also correlated with lifestyle-related diseases. A markedly reduced diversity and compositional change of the gut microbiota has been linked to obesity and insulin resistance (63-65). A high fat diet was shown to affect the composition of gut microbiota through modifying the expression of bacterial genes responsible for nutrient uptake and adaptation to environmental change in the host intestine (66). A dysbiotic intestinal environment contributes to the pathogenesis of liver diseases (67, 68). Also, Schnabl and Brenner (68) showed a correlation between altered intestinal microbiota and nonalcoholic fatty liver disease/steatohepatitis. Furthermore, absorbed bacterial metabolites have the potential to systemically enhance the development of these metabolic diseases. Treatment with prebiotics or probiotics has been reported to reverse high fat diet-induced metabolic disorders through an increase of *Akkermansia muciniphila* (69) and to suppress nonalcoholic steatohepatitis (70) in a rodent model.

7. CONCLUDING REMARKS

The lifestyle of the average person, especially in regards to dietary habits, has changed dramatically over the past several decades. A parallel increase in patients with inflammatory bowel diseases, irritable bowel disease, allergy, autoimmune diseases, and metabolic diseases like type 2 diabetes and fatty liver disease has occurred. The role of the intestinal microbiota in human health is apparent now because of research that connects changes of the gut microbiota with the risk of diseases. The diversity and total amount of intestinal microbiota is continuously influenced by environmental bacteria, dietary habits, exercises, stress, aging, intake of probiotics/prebiotics, medical treatment with antibiotics or other drugs, host immune system, and the person's genetic background (63, 71). While modification of the intestinal microbiota and intestinal environment including mucosal immune system could affect the risk of many kinds of diseases, the strongest influence might occur during early stage of life as described elsewhere (63, 72-74). Despite our understanding that the microbiota is a critical part of health, it remains to be discovered what type of microbiota should be present at different stages of life. Further studies should focus on how to maintain a healthy composition of the intestinal microbiota.

Although 16S rDNA-targeting PCR and sequencing analyses yielded information about microbiota composition at the genus or species level, variation between individuals was too large to find a functional difference between healthy and disease states. Improvement of current sequencing techniques would allow better analysis of the complexity of the gut microbiota. Alternatively, we could look for a change in specific bacterial gene functions in diseased persons. Large scale metagenomics studies of disease and healthy states have begun to point to the kinds of bacteria that correlate with risk of disease. The NIH "Human Microbiome Project" has steadily been clarifying the relationship between intestinal microbiota and human health by this method (75). Recently, a different

metagenome-wide association study reported taxonomic and functional characterization of gut microbiota in type 2 diabetes, and they correlated the disease with lack of bacterial richness and metabolic markers (76, 77). Additionally, bacterial metabolites, which are affected by dietary components, should be analyzed because these products from dietary can affect human health similar to some metabolic diseases. Careful studies to clarify the roles of intestinal microbiota in human health will bring us great progress in treatment and prevention of many kinds of diseases.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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