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ORIGINAL ARTICLE

The effect of *Clostridium butyricum* MIYAIRI on the prevention of pouchitis and alteration of the microbiota profile in patients with ulcerative colitis

Asuka Yasueda[1] · Tsunekazu Mizushima[2,3] · Riichiro Nezu[4] · Ryoko Sumi[5] · Mamoru Tanaka[6] · Junichi Nishimura[2] · Yasuyuki Kai[7] · Masaki Hirota[8] · Hideki Osawa[2] · Kiyokazu Nakajima[9] · Masaki Mori[2] · Toshinori Ito[1]

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Abstract
Purpose Ulcerative colitis (UC) is a chronic, relapsing, and refractory disorder of the intestine. Total proctocolectomy with ileal pouch anal anastomosis (IPAA) is the preferred and standard surgical procedure for patients' refractory to medical therapy. Pouchitis is one of the most common long-term complications after IPAA. In the present study, the safety and efficacy of *Clostridium butyricum* MIYAIRI (CBM) as a probiotic were examined.
Methods A randomized and placebo-controlled study was performed. Seventeen patients were recruited from 2007 to 2013. Nine tablets of MIYA-BM® or placebo were orally administered once daily. The cumulative pouchitis-free survival, pouch condition (using the modified pouch disease activity index), and blood parameters were evaluated. A fecal sample analysis was also performed.
Results Subjects were randomly allocated to receive MIYA-BM or placebo (9 and 8 subjects, respectively). One subject in the MIYA-BM group and four subjects in the placebo group developed pouchitis. No side effects occurred in either group. Characteristic intestinal flora was observed in each group.
Conclusions Our results suggest that probiotic therapy with CBM achieved favorable results with minimal side effects and might be a useful complementary therapy for the prevention of pouchitis in patients with UC who have undergone IPAA.

Keywords Pouchitis · Probiotics · *Clostridium butyricum* MIYAIRI · Ulcerative colitis · IPAA

Abbreviations

Alb	Albumin
AST	Aspartate aminotransferase

✉ Toshinori Ito
juki@cam.med.osaka-u.ac.jp

1 Department of Integrative Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

2 Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

3 Department of Therapeutics for Inflammatory Bowel Diseases, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

4 Department of Surgery, Nishinomiya Municipal Central Hospital, 8-24 Hayashidacho, Nishinomiya, Hyogo 663-8014, Japan

5 Division of Nutrition and Medical Engineering, Center for Advanced Medical Engineering and Informatics, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

6 Miyarisan Pharmaceutical Co. Ltd., 102-15 Sakaki, Hanishina, Nagano 389-0682, Japan

7 Gastroenterological Surgery, Hanwasumiyoshi General Hospital, 3-2-9 Minami Sumiyoshi, Sumiyoshi-ku, Osaka 558-0041, Japan

8 Gastroenterological Surgery, Osaka Rosai Hospital, 1179-3 Nagasone-cho, Kita-ku, Sakai, Osaka 591-8025, Japan

9 Division of Collaborative Research for Next Generation Endoscopic Intervention, Center for Advanced Medical Engineering and Informatics, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

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ALT	Alanine aminotransferase
BUN	Blood urea nitrogen
CBM	*Clostridium butyricum* MIYAIRI
CD	Crohn's disease
Cre	Creatinine
CRP	C-reactive protein
CTCAE v.4.0	Common terminology criteria for adverse events version 4.0
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
FAP	Familial adenomatous polyposis syndrome
Hb	Hemoglobin
IBD	Inflammatory bowel disease
IPAA	Abdominal colectomy with ileal pouch anal anastomosis
ITT	Intention to treat
IF-γ	Interferon γ
IL-10	Interleukin 10
mPDAI	Modified pouchitis disease activity index
NF-κB	Nuclear factor kappa B
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction
PDAI	Pouchitis disease activity index
RBP	Retinol-binding proteins
rRNA	Ribosomal ribonucleic acid
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulfate
TTR	Transthyretin
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor α
TP	Total protein
T-RFLP	Terminal restriction fragment polymorphism
UC	Ulcerative colitis
WBC	White blood cell
6-FAM	6′-Carboxyfluorescein
% AUC	% Area under the curve

Introduction

Ulcerative colitis (UC) and Crohn's disease, generally known as inflammatory bowel disease (IBD), are chronic, relapsing, and refractory disorders of the intestine [1, 2]. Though the precise mechanism remains unclear, dietary ingredients and commensal bacteria can trigger the development of IBD [3, 4]. Patients with UC usually require biphasic pharmacologic therapy composed of immunomodulatory drug induction treatments in the acute phase followed by maintenance agents in the clinical phase. Total proctocolectomy with ileal pouch anal anastomosis (IPAA) is the preferred and standard surgical procedure for patients' refractory to medical therapy [5]. Pouchitis, defined as idiopathic inflammation of the ileal pouch mucosa, is one of the most common long-term complications after IPAA [6]. Despite the improved quality of life in patients who have undergone IPAA, some patients suffer from refractory pouchitis [7, 8]. An investigation by Uchino et al. [9] and Hashimoto et al. [10] revealed that approximately 10 % of Japanese patients with UC developed pouchitis after surgery. The pathogenesis of pouchitis is not completely understood. Proposed etiologies include changes in the enteral environment, such as bacterial overgrowth, dysbiosis, and short-chain fatty acid deficiency. In addition, other causes such as genetic susceptibility and ischemic complications of the surgical procedure have also been proposed [6, 11]. Antibiotic therapy with metronidazole or ciprofloxacin is frequently effective in treating pouchitis. However, despite effective treatment, pouchitis often relapses when the therapy concludes. If this occurs, then the patients have to continue drug therapy, which is not an advisable long-term solution [12].

It has been demonstrated that probiotics can prevent pouchitis [12–14]. Previous studies of probiotics used a combination of bacteria such as *Lactobacilli*, *Bifidobacterium*, and *Streptococcus salivarius* [13–15]. Many of the previous studies reported that probiotic therapy was effective in preventing the onset of pouchitis and maintaining remission postoperatively. It is difficult to determine which type of bacteria is effective in preventing or treating pouchitis. Furthermore, it is difficult to observe the friability of the bacteria normally present in the ileal pouch.

In the present study, spore-forming bacteria *Clostridium butyricum* MIYAIRI (CBM) were studied. These bacteria can reach the target location in the bowel without being affected by digestive juices and grow extensively upon reaching the location. In this study, we examined the safety and efficacy of CBM treatment in the prevention of pouchitis. Furthermore, we investigated how CBM contributed to changes in the intestinal flora and whether or not these changes contributed to the prevention of pouchitis. These endpoints were investigated using a randomized and placebo-controlled trial.

Materials and methods

This study was approved by the ethical committee of Osaka University Hospital in 2006. A total of 17 patients with UC who were scheduled for IPAA surgery were recruited in this study between 2007 and 2013. Written informed consent was obtained from all subjects. Patients who had already developed pouchitis were excluded. Among those enrolled, 10 subjects were managed at the Osaka University Hospital and seven were managed at the Osaka Rosai Hospital.

Therapy protocol: MIYA-BM® (Miyarisan Pharmaceutical Co., Ltd., Tokyo, Japan), which is commercially available and contains 20 mg of CBM, was purchased. The placebo was composed of lactose and was provided by Miyarisan Pharmaceutical Co., Ltd. in the same form as the MIYA-BM tablets. Three tablets of MIYA-BM or placebo were orally administered three times per day. The taste, smell, and shape of the active drug were not readily identifiable. The subjects received either MIYA-BM or placebo for 24 months after hospital discharge.

Study design

This study was a randomized and placebo-controlled study. Before surgery, the subjects were randomly allocated to the MIYA-BM group or the placebo group. For the subjects who had a single-stage operation (IPAA without protective ileostomy), the therapy was administered after the operation. For the subjects who underwent a staged operation (IPAA with protective ileostomy), the therapy was administered after the ileostomy closure operation.

Assessment

In subjects with UC who undergo IPAA, the pouchitis disease active index (PDAI) score is a good index for the diagnosis of pouchitis [16]. However, PDAI scoring requires the assessment of clinical symptoms, an endoscopic examination, and a histological examination. These assessments impose a substantial burden on the patients. Shen et al. [17] proposed a simplified approach to the diagnosis of pouchitis. The omission of an endoscopic biopsy and a histological examination from the PDAI simplifies the diagnostic criteria for pouchitis, while providing equivalent sensitivity and specificity [17]. This simplified scoring system is called the modified PDAI (mPDAI). In the present study, the pouch condition was evaluated using the mPDAI. An endoscopic examination of the ileal pouch was planned at 3 (point 1), 6 (point 2), 12 (point 3), 18 (point 4), and 24 (point 5) months. In cases in which the patient's consent could not be obtained, the endoscopic examination was omitted. However, endoscopy was performed in all cases if pouchitis was suspected from their symptoms. According to the criteria of Shen et al., patients with a total mPDAI of $\geq$4 were diagnosed as having pouchitis [17]. Blood tests and a fecal sample analysis [16] were performed at baseline (point 0: before therapy) and points 1–5.

The white blood cell count (WBC), hemoglobin (Hb), and serum levels of C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine (Cre) were evaluated in the blood tests. Abnormalities of these parameters were graded according to the common terminology criteria for adverse events version 4.0 (CTCAE v4.0) [18].

A fecal sample analysis was performed at points 0–5. The present study aimed to verify the relationship between changes in the intestinal environment caused by the administration of probiotics and pouchitis development. For this purpose, the method of fecal sample analysis was as follows. The stool samples of subjects treated at Osaka University Hospital were collected using a stool test kit, soaked in DNA buffer solution, and sent to the Miyarisan Pharmaceutical Co., Ltd laboratory. The samples were immediately stored at −20 °C until the evaluation of bacterial microflora. Subjects treated at Osaka Rosai Hospital had their fecal samples collected using a different method. The fecal samples were collected during the endoscopic examination, immediately stored at −20 °C, and sent to the laboratory above. Thus, the distribution of bacteria could be slightly different between the hospitals due to differences in the fecal collection methods.

The method of deoxyribonucleic acid (DNA) extraction was as follows. The fecal samples were disrupted with glass beads using FastPrep-24 (MP Biomedicals, Irvine, CA, USA), and DNA was extracted with phenol. The extracted DNA was purified using a High Pure polymerase chain reaction (PCR) Kit (Roche Diagnostics, Indianapolis, IN, USA), and the final concentration of the DNA sample was adjusted for the analysis. A PCR amplification and terminal restriction fragment polymorphism (T-RFLP) analysis was then performed. The primers used for PCR amplification of the 16S rRNA gene were 27F labeled with 6-FAM and 1492R. PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics, Penzberg, Germany) and were digested with *Hha*I and *Msp*I. The digested products were analyzed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster, CA, USA). The fragment sizes of T-RFs were estimated using the GeneMapper Software program (Applied Biosystems). Major T-RFs similar in size were summarized to operational taxonomic units (OTUs). The OTUs were quantified as the percentage value of an individual OTU per total OTU area, and this was expressed as the percentage area under the curve (% AUC).

Despite carrying out the above analysis, the data of seven subjects from Osaka Rosai Hospital did not have high reproducibility. To enhance the integrity of these data for analysis and to avoid data mismatch between the methods of sample collection, only the data of the 9 subjects treated at Osaka University Hospital were evaluated.

Statistical analysis

Numerical data are presented as the median (range). All statistical analyses were carried out using the JMP Pro10 software program (SAS Institute Inc., Cary, NC, USA). Numerical data comparing the two groups were assessed

using a Wilcoxon rank-sum test. A comparison analysis of the anteroposterior value was carried out using a paired *t* test, and the available data of each subject at the last point were used. The occurrence of pouchitis and dropout were estimated as a function of time using the Kaplan–Meier method and the Chi-square test. Cluster analyses were performed on the basis of the T-RFLP patterns digested with *Hha*I or *Msp*I. The distances were calculated to determine any similarity among the samples and were represented graphically in a dendrogram [19–21]. Ward's method was used for a clustering analysis and generating the dendrogram. With all analyses, a p value of <0.05 was considered to be statistically significant.

Results

Patient characteristics

Seventeen patients were recruited and were eligible for this trial. Nine patients were randomly allocated to receive MIYA-BM and eight patients to receive placebo. Two patients in the MIYA-BM group withdrew from the study at the 18- and 2-month points due to difficulty in taking the medication (Dropout-1 and Dropout-2, respectively). According to the concept of intention to treat, the analysis of the data, including those of Dropout-1 and 2, was performed regarding the primary endpoint (rate of pouchitis development). However, Dropout-2 was excluded from other analyses, such as mPDAI, blood tests analysis, and fecal sample analysis, because the majority of the data (blood assessment, fecal sample analysis, and endoscopic findings) could not be obtained. The characteristics of the subjects are shown in Table 1. No difference was observed between both groups regarding sex, age, type of UC, operative indication [5, 22], and anastomosis. These factors thus appeared to be unlikely to affect the clinical results. Regarding disease duration, a significant difference was observed between the groups ($p = 0.03$). However, the difference was not considered disadvantageous to the placebo group.

Development of pouchitis and the mPDAI score

One subject in the MIYA-BM group and four subjects in the placebo group developed pouchitis. The Kaplan–Meier plot shows the time taken to develop pouchitis or to withdraw from the trial in subjects from both groups over the 24-month follow-up period (Fig. 1). Thus, 50 % of the subjects in the placebo group and 11 % of the subjects in the MIYA-BM group developed pouchitis. The subjects in the placebo group tended to have an increased risk of developing pouchitis compared with those in the MIYA-BM group. However, pouchitis development did not differ significantly between the groups according to the Kaplan–Meier plot ($p = 0.30$) or the Chi-square test ($p = 0.07$).

The mPDAI score was calculated for 16 subjects. The worst (maximum) mPDAI score was evaluated at each

Table 1 Baseline clinical characteristics in the MIYA-BM and placebo groups

	MIYA-BM ($n = 9$)	Placebo ($n = 8$)	p value
Sex (M/F)	5/4	4/4	0.80
Mean age (years, recruited point)	47 (25)	34 (48)	0.20
Type of UC			
Total colitis	8	7	0.92
Right-sided or segmental colitis	0	0	
Left-sided colitis	1	1	
Proctitis	0	0	
Operative indication			
Fulminant colitis	2	2	0.58
Unresponsive to medical management	4	5	
Dysplasia or cancer	3	1	
Anastomosis			
Stapled	8	7	0.92
Hand-sewn	1	1	
Disease duration (months)	60 (288)	18 (186)	0.03

Median (range)

Characteristic data comparing the two groups were analyzed using the Wilcoxon rank-sum test. Type of UC, operative indication, and anastomosis were analyzed using Fisher's exact test

Significant differences were observed during the disease duration only

UC ulcerative colitis

point for each subject. The median mPDAI value of 2 (seven subjects) at the worst median score in the MIYA-BM group tended to be lower than that of 3 (six subjects) in the placebo group ($p = 0.17$). The mPDAI scores, endoscopic findings, and clinical scores are shown in Fig. 2.

Blood test

The renal and liver function test results were assessed using the common terminology criteria for adverse events version 4.0 (CTCAE v4.0) [18]. As shown in Table 2, the serum levels of AST and ALT showed significant differences between the groups at the worst point analysis. However, these data were not outliers threatening the safety. The serum CRP levels significantly decreased after therapy, even at the worst point analysis, in the MIYA-BM group only ($p = 0.003$). No significant differences were observed between the groups regarding the serum total proteins. The serum albumin levels significantly increased after therapy, even at the worst point analysis, in the MIYA-BM group only ($p = 0.03$). Regarding safety, no side effects occurred in either groups, and the subjects did not experience difficulty in taking nine tablets daily.

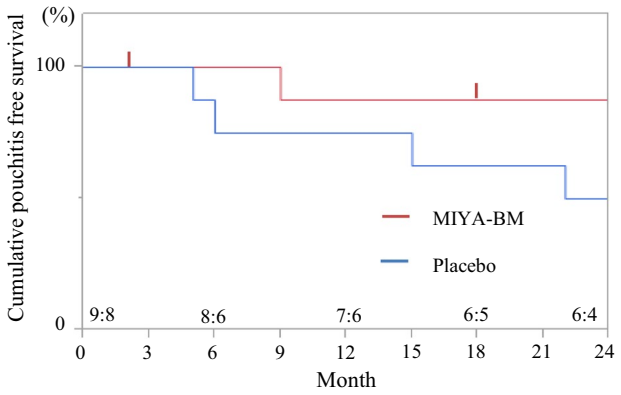

Fig. 1 Cumulative pouchitis-free survival curves. Kaplan–Meier cumulative pouchitis-free survival curves show the time taken to develop pouchitis or to withdraw from the trial in subjects from both groups over a 24-month follow-up period

Fecal microbiota

According to the analyses of fecal bacterial flora, fecal microbial diversities were compared by means of a dendrogram constructed using a minimal variance algorithm, as shown in Fig. 3. Four dendrograms were constructed. Flora trend analyses were performed before and after digestion with *Hha*I(a) and *Msp*I(c) and *Hha*I(b) and *Msp*I(d), respectively. The setting of similarity generated four major clusters, and each cluster was color-coded. This analysis showed the similarities in each group after digestion, although they were not identified before therapy, especially after *Msp*I digestion.

Representative operational taxonomic units (OTUs) after digestion with *Hha*I and *Msp*I digestion are shown in Fig. 4. As shown in Fig. 4a, b, the associated OTU digested with *Hha*I indicated that the levels of the *Clostridium coccoides* group (colored orange) tended to increase after therapy in the placebo groups. The proportion of the *Enterococcus* group (colored green) in both groups tended to decrease after therapy as compared with that before therapy. Similarly, we analyzed the T-RFLP patterns resulting from digestion with *Msp*I (Fig. 4c, d), and the associated OTU indicated that the levels of the *Clostridium coccoides* group were significantly increased after therapy in the placebo group ($p = 0.02$). The levels of the *Escherichia* subgroup (colored bright blue) were significantly decreased in the MIYA-BM group after therapy compared with those before therapy ($p = 0.04$). The levels of the *Bifidobacterium* (colored purple) and *Bacteroides* (colored blue) groups were detected to be higher in the placebo group compared with the MIYA-BM group after the therapy.

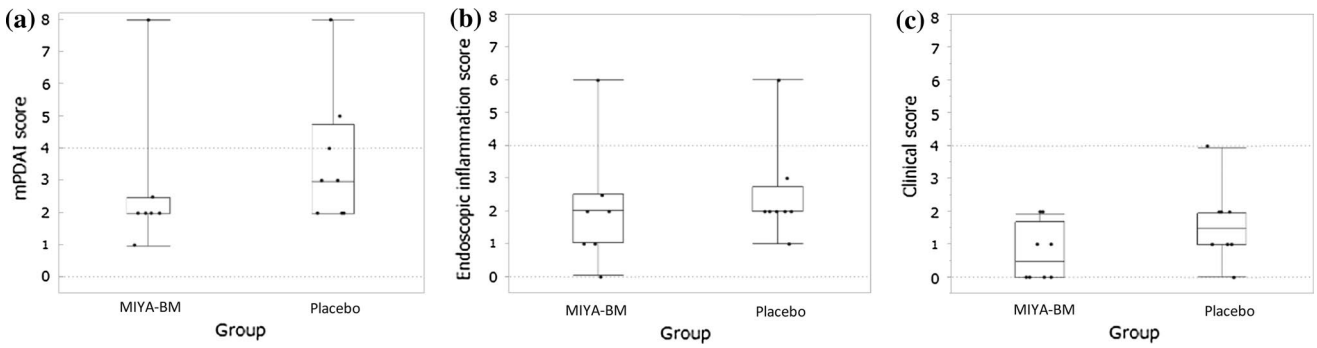

Fig. 2 Plot and box chart of the mPDAI scores. The highest mPDAI scores of each subject in both groups during the intervention period. **a** mPDAI score. **b** Endoscopic inflammation score. **c** Clinical score. The *points* in the figure indicate the scores. The segment inside the *rectangle* shows the median. *Whiskers above* and *below* the *box* show the minimum and maximum data

Table 2 Blood test results before treatment and the worst values

	MIYA-BM	Placebo	*p* value
Alb			
Before treatment	3.7 (0.7)	3.8 (0.4)	0.72
Worst	4.15 (1)	3.9 (0.7)	0.52
TP			
Before treatment	7.35 (0.8)	6.8 (1.4)	0.26
Worst	7.15 (7.6)	7.05 (1.5)	0.55
WBC			
Before treatment	5.86 (2.1)	5.36 (2.76)	0.40
Worst	6.32 (3.3)	6.48 (8.34)	0.75
Hb			
Before treatment	11.4 (3.4)	12 (3.7)	0.31
Worst	13.5 (2.3)	13.3 (2.2)	0.75
CRP			
Before treatment	0.43 (1.14)	0.54 (3.36)	0.63
Worst	0.16 (2.51)	0.49 (5.6)	0.63
AST			
Before treatment	18.5 (18)	19.5 (15)	0.79
Worst	26.5 (16)	19 (23)	0.02
ALT			
Before treatment	24 (59)	22 (26)	0.87
Worst	27 (37)	13 (19)	0.03
BUN			
Before treatment	9.5 (9)	12.5 (13)	0.67
Worst	16 (13)	13.5 (5)	0.59
Cre			
Before treatment	0.7 (0.2)	0.61 (0.73)	0.63
Worst	0.8 (0.17)	0.7 (0.51)	0.75

Median (range)

The blood test results before the treatment and the worst values during the observation period. A statistical analysis between the groups was performed using the Wilcoxon rank-sum test. Only the serum levels of AST and ALT showed significant differences between the groups. A p value of <0.05 was considered to be statistically significant

Alb albumin, *TP* total protein, *WBC* white blood cell, *Hb* hemoglobin, *CRP* C-reactive protein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *BUN* blood urea nitrogen, *Cre* creatinine

As shown in Fig. 5a, b, the proportion of obligate anaerobes (colored green) tended to increase in the MIYA-BM group after therapy compared with that before therapy. Figure 5c, d indicates that the levels of facultative anaerobes (colored blue) significantly increased in the MIYA-BM group after therapy compared with those before therapy ($p = 0.04$).

Discussion

Probiotics are live and nonpathogenic bacteria which regulate the intestinal function by improving the intraluminal environment. Recently, changes in the bacterial flora have been demonstrated in IBD [21]. For many years, probiotic therapy remained in the non-academic shadow of complementary and alternative medicine. In the modern era, however, as a result of many advances in medicine, an overwhelming amount of knowledge on the biological and therapeutic effectiveness of probiotics has been accumulated [19]. Ohigashi et al. [23] reported that probiotic therapy could be effective for improvements in the functional outcome and QOL after colorectal resection.

Many studies have focused on probiotics for the treatment of patients with UC who develop pouchitis after surgery. Mimura et al. investigated the effectiveness of probiotics in the maintenance of remission using probiotics of VSL #3 that consisted of eight strains of live lyophilized bacteria. They conducted a placebo-controlled RCT for 36 patients with pouchitis (VSL #3, 20 patients; placebo, 16 patients). They investigated the maintenance of remission after pouchitis development. In their results, remission was maintained at 1 year in 85 % of the patients in the VSL #3 group compared with only 6 % patients in the placebo group ($p < 0.0001$) [12]. Similarly, Gionchetti et al. [13, 14] conducted placebo-based RCTs for patients who underwent IPAA. They investigated the effects of probiotic therapy on the prevention of pouchitis development. In their results, only 15 % of the patients treated with VSL #3 relapsed within 9 months, compared with 100 % of the patients treated with placebo ($p < 0.001$) [13]. From the above results, VSL #3 treatment was effective in preventing pouchitis and maintaining remission after its administration. The authors described the following mechanism for the prevention of pouchitis. In pouchitis, the tissue levels of tumor necrosis factor α (TNF-α), interferon-γ (IFN-γ), inducible nitric oxide synthase, and matrix metalloproteinases 2 and 9 increase, while the tissue level of interleukin-10 decreases. VSL #3 treatment contributes to the normalization of the colonic physiological function and barrier integrity, in conjunction with the reduction of TNFα and IF-γ in the mucosa. Furthermore, the epithelial barrier function is enhanced due to exposure to a proteinaceous soluble factor secreted by the bacteria in VSL #3.

CBM is a spore-forming bacterial strain used safely in Japan for over 40 years. In the present study, CBM was able to reach the pouch in a living state without being affected by the digestive juices. MIYA-BM is clinically targeted for conditions such as diarrhea, constipation, and other intestinal symptoms associated with the disruption of microflora of the gastrointestinal tract [24]. Although it was not a placebo-controlled trial, this study demonstrated that CBM inhibits the growth of putrefying bacteria and increases the levels of beneficial bacteria, especially bifidobacteria and lactobacilli [25]. However, in the present study, we reported that *Bifidobacterium* were detected at

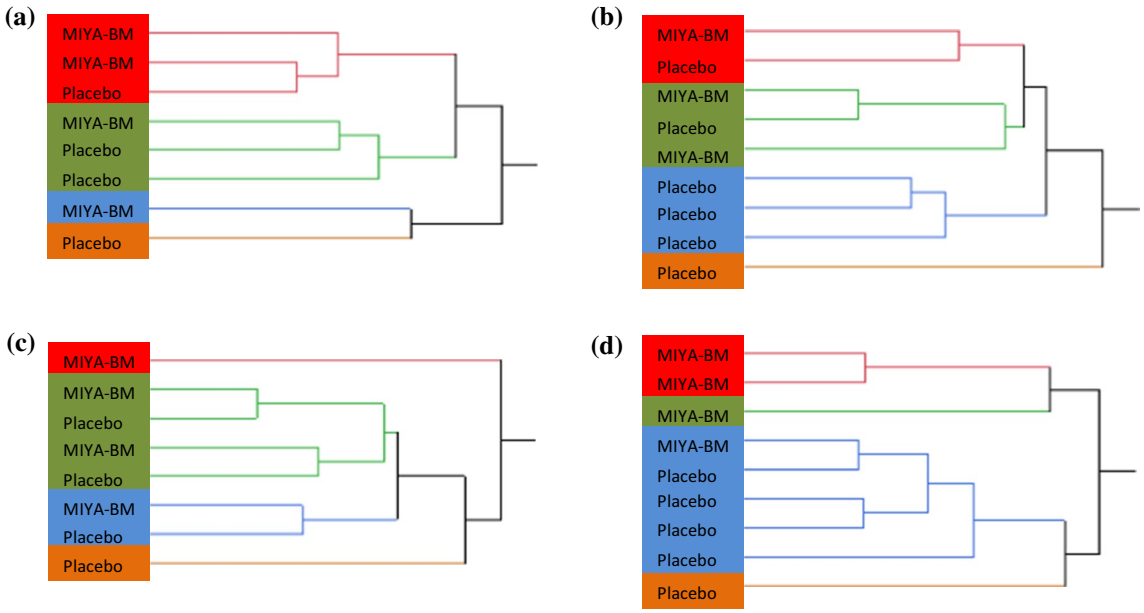

Fig. 3 Dendrogram of the fecal microbiota community. Dendrograms of the fecal microbiota community of each subject at point 0 and after therapy. Setting of similarity generated four major clusters, and each cluster is *color*-coded. **a** Dendrogram of fecal microbiota T-RFLP patterns caused by digestion with *Hha*I at point 0. **b** Dendrogram of fecal microbiota T-RFLP patterns caused by digestion with *Hha*I after therapy. **c** Dendrogram of fecal microbiota T-RFLP patterns caused by digestion with *Msp*I at point 0. **d** Dendrogram of fecal microbiota T-RFLP patterns caused by digestion with *Msp*I after therapy

increased levels in the placebo group compared with the MIYA-BM group after therapy. Although specific reasons cannot be identified, the potential of the placebo composition could not be ruled out. The placebo used in the present study included 20 mg of lactose instead of 20 mg of CBM. It is known that the lactose intake is associated with *Bifidobacterium* in the intestinal flora [26], and it is the predominant intestinal flora of breast-feeding infants [27]. Furthermore, CBM produces short-chain fatty acids (SCFAs), such as butyrate, acetate, and propionate. There are many reports that have demonstrated that SCFAs have potentially beneficial effects, such as proliferative effects on the intestinal mucosal epithelium and anti-inflammatory effects [28].

Our present study aimed to demonstrate whether MIYA-BM is effective for the prevention of developing pouchitis in patients with total proctocolectomy with IPAA [29]. The patients could maintain a high QOL with this therapy. We investigated not only the incidence of pouchitis, but also the changes in the intestinal flora before and after treatment.

In the present study, the levels of the *Escherichia* subgroup were significantly decreased in the MIYA-BM group after therapy compared with those before therapy. There are some reports regarding the relationship between the levels of *Escherichia coli* (*E. coli*) and gastrointestinal tract inflammation in patients with IBD [30, 31]. Pilarczyk-Zurek et al. [32] reported that the levels of *E. coli* were significantly increased in the inflammatory mucosa of patients with UC. Furthermore, they examined the association between the iron ion and *E. coli*. An increase in the *E. coli* populations was dependent on many factors, such as biofilm formation, the synthesis of enzymes to catalyze the breakdown of reactive oxygen species (ROS), the use of supportive mechanisms allowing absorption of iron ions from the environment, and the ability to acquire iron ions using hemoglobin from lysed erythrocytes. One factor that increases *E. coli* populations is an enhanced availability of iron ions in the intestinal tract of patients with UC [32, 33]. Furthermore, an increased *E. coli* population can bind free iron ions using siderophores and store iron intracellularly, which allows *E. coli* to inhibit the Fenton reaction by the elimination of iron ions and prevent ROS formation, reducing damage to the host tissue. However, the results of the present study show that the *Escherichia* subgroup was significantly decreased in the MIYA-BM group alone. Previous reports on the relationship between CBM and iron ions were not identified. The therapy administered in the present study might have caused a decreased availability of iron ions, which led to decreased *E. coli* populations in the pouch. The decreased *E. coli* populations may have caused a decreased expression of some factors involved in inflammation, which led to the onset of pouchitis. One of the limitations of the present study was that the levels of iron ions in the pouch were not measured. Further studies should include these analyses to confirm the above theory.

The effect of the butyrate mechanism on the suppression of intestinal inflammation has been reported [34].

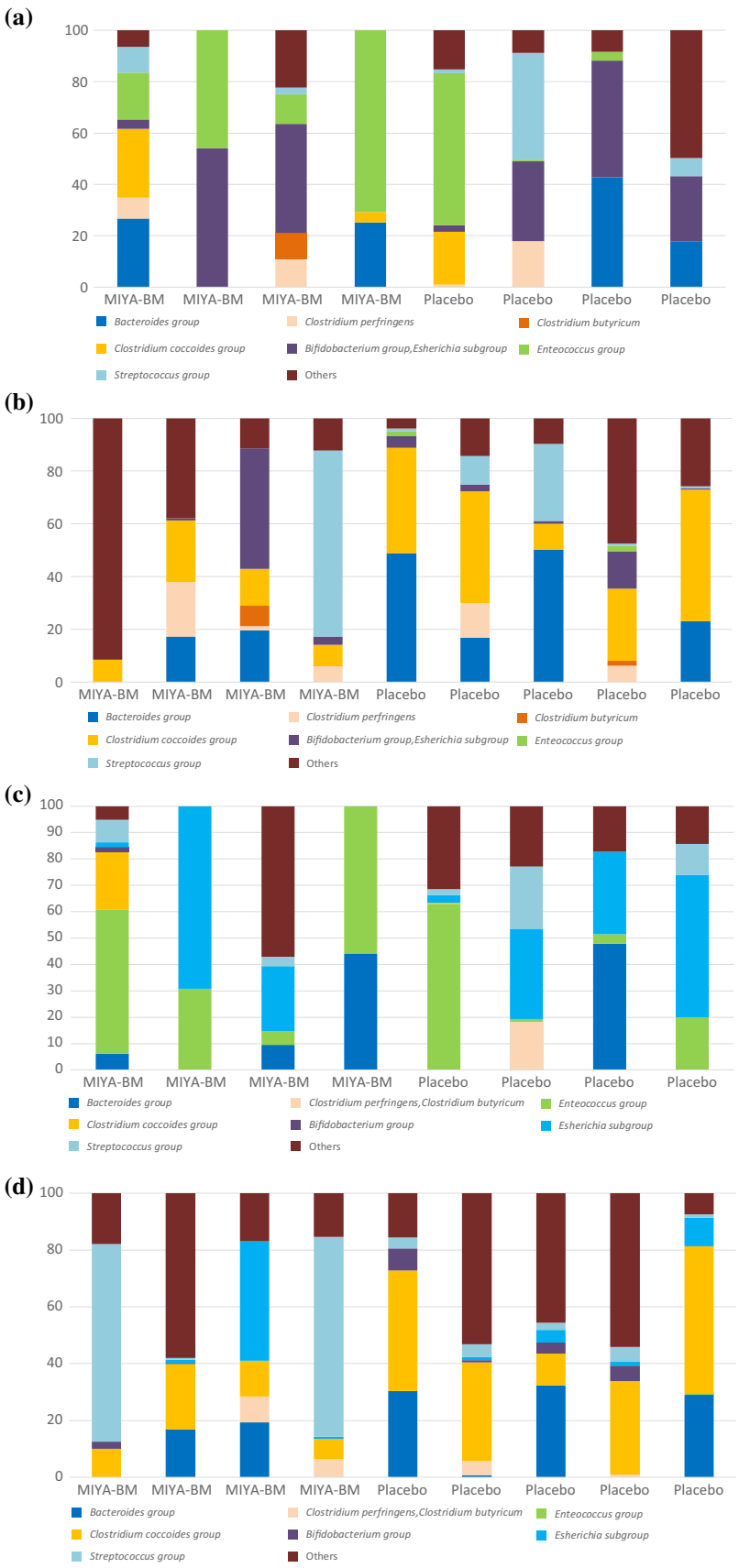

Fig. 4 Representative OTUs of fecal microbiota. Representative fecal microbiota OTUs of each sample. Unknown bacteria are compiled in the category "Others." **a** OTU patterns of fecal microbiota digested with *Hha*I at point 0. **b** OTU patterns of fecal microbiota digested with *Hha*I after therapy. **c** OTU patterns of fecal microbiota digested with *Msp*I at point 0. **d** OTU patterns of fecal microbiota digested with *Msp*I after therapy

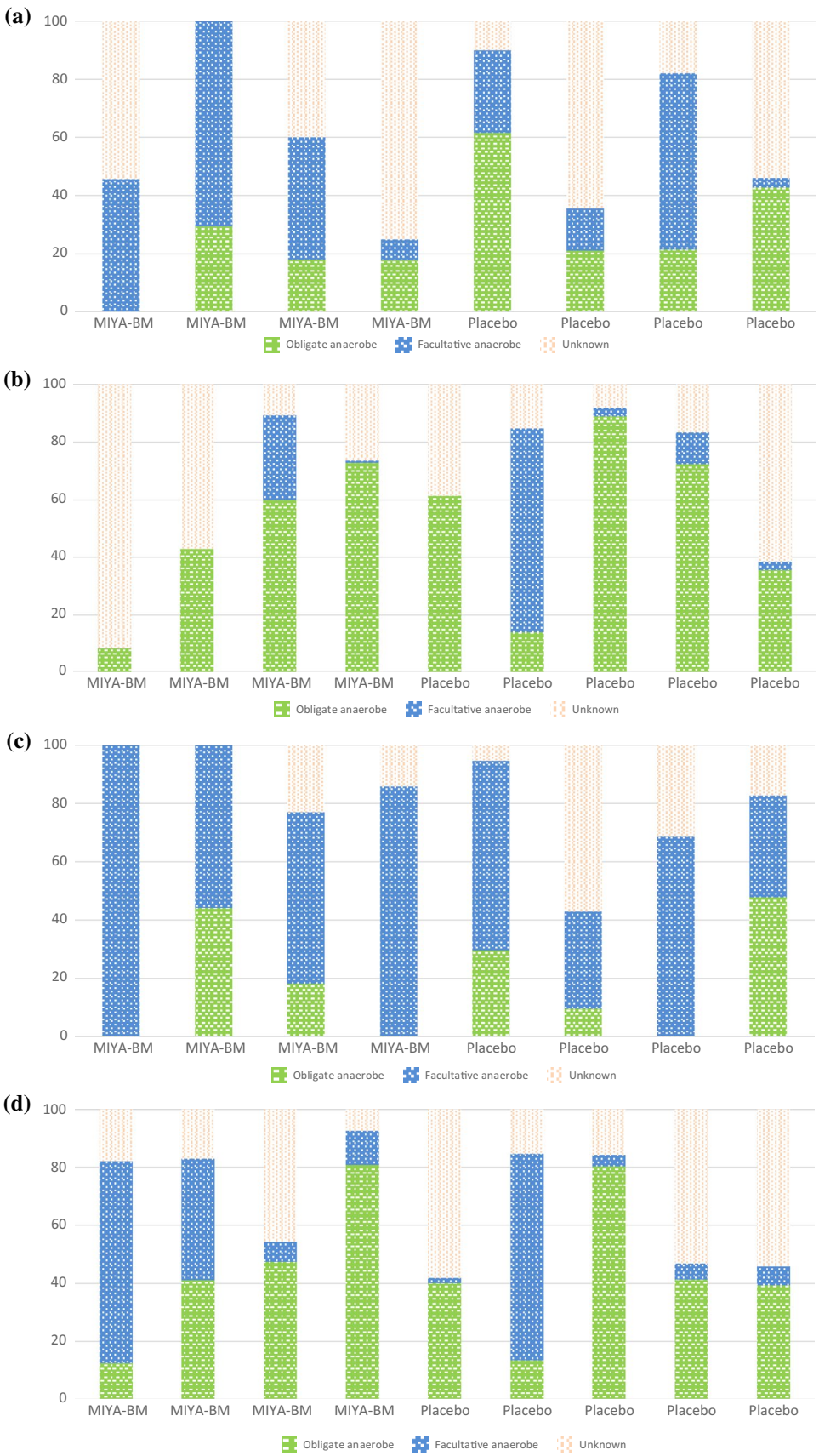

Fig. 5 Representative OTUs indicated by the type of bacteria. Representative bacteria types for each sample. Each type of bacteria is divided into two groups, obligate anaerobes and facultative anaerobes. The bacteria classified in other and unknown categories are shown in "unknown." **a** Bacteria patterns of fecal microbiota digested with *Hha*I at point 0. **b** Bacteria patterns of fecal microbiota digested with *Hha*I after therapy. **c** Bacteria patterns of fecal microbiota digested with *Msp*I at point 0. **d** Bacteria patterns of fecal microbiota digested with *Msp*I after therapy

Clostridium butyricum produces organic acids, such as acetic acid and butyric acid, which reduce the pH and inhibit the growth of pathogenic bacteria in the intestinal tract [35, 36]. Furthermore, Furusawa et al. reported that butyrate is linked to the commensal microbe-mediated induction of functional Treg cells in the colonic mucosa and mediates a host–microbial crosstalk for the establishment of gut immune homeostasis [37]. A relationship between butyrate and the intestinal flora in patients with UC has been suggested in some studies [38, 39]. There are two mechanisms which may explain the effectiveness of butyrate. First, butyrate reduces nuclear factor kappa B (NF-κB) translocation of macrophages in the lamina propria [40] and also mediates TNF-α induced apoptosis [41]. Another mechanism is the inhibition of PU.1 [42, 43], which is a tissue-specific transcription factor, resulting in the downregulation of the Toll-like receptor 4 (TLR4) expression [44]. Regarding the association of pouchitis development and upregulation of TLR4, it was recently reported that constant upregulation of TLR4 in the pouch mucosa might represent a hyperresponse to commensal intraluminal bacteria, thus resulting in mucosal inflammation [45]. In the present study, as shown in Figs. 3, 4 and 5, characteristic intestinal flora was observed in each group.

Regarding the limitations of the present investigation, the T-RFLP method analysis could not describe the intestinal flora in detail. Future detailed analyses of the intestinal flora using DNA-sequencing analysis are recommended [6]. In addition, the present investigation was performed with a small sample size, which was insufficient to completely elucidate the effects of probiotic therapy using CBM for the prevention of pouchitis.

In conclusion, our results suggested that probiotic therapy using CBM might be a useful complementary therapy with minimal side effects for the prevention of pouchitis in patients with UC who have undergone IPAA. Future larger scale investigations can be conducted according to the results of the current study.

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Compliance with ethical standards

Conflict of interest This work was mainly carried out by funds from the investigators themselves and was partly supported by Miyarisan Pharmaceutical Co., Ltd. They provided the placebo and the meeting space and analyzed fecal microbiota. Other costs related to this research were covered by the Department of Integrative Medicine, Osaka University Graduate School of Medicine. This laboratory is supported by donated funds and is managed by a fund from Amino Up Chemical Co., Ltd. Mamoru Tanaka is an employee of Miyarisan Pharmaceutical Co. Ltd. and supervised the measurement of the fecal bacterial flora. For the remaining authors, no conflicts were declared. However, none of the sponsors had any control over the research, writing, or publication of this work.

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