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Construction of a model culture system of human colonic microbiota to detect decreased *Lachnospiraceae* abundance and butyrogenesis in the feces of ulcerative colitis patients

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Keywords

Ulcerative colitis, Model culture system, Microbiota, Butyrate, Probiotics

Abbreviations

UC ulcerative colitis; HS healthy subject; KUHIMM Kobe University Human Intestinal Microbiota Model; NGS next-generation sequencing; SCFA short chain fatty acid; 5-ASA 5-aminosalicylic acid; OTU operational taxonomic unit; PCoA principal coordinate analysis; HPLC high-performance liquid chromatography; MANOVA multivariate analysis of variance.

Abstract

Compositional alteration of the gut microbiota is associated with ulcerative colitis (UC). We established a model culture system for the *in vitro* human colonic microbiota of UC, which will be helpful for determining medical interventions for the disease. 16S rRNA sequencing confirmed that UC models successfully developed from the fecal inoculum and retained the bacterial species biodiversity of UC feces. UC models closely reproduced the microbial components, although not completely, and successfully preserved distinct clusters from healthy subjects (HS), as observed in the feces. The relative abundance of bacteria belonging to family *Lachnospiraceae* was significantly decreased in the UC models compared to in the HS, as observed in the feces. Our system detected significantly lower butyrogenesis in the UC models than in HS, correlating with the decreased abundance of *Lachnospiraceae*. Interestingly, the relative abundance of *Lachnospiraceae* did not correlate with disease activity (defined as partial Mayo score), suggesting that *Lachnospiraceae* persisted in UC patients at a decreased level, irrespective of altered disease activity. Moreover, our system showed that administration of *Clostridium butyricum* MIYAIRI restored butyrogenesis in the UC model. Our model detects deregulation in the intestinal environment in UC patients and may be useful for simulating the effect of probiotics.

1. Introduction

The human gut microbiota comprises numerous microorganisms, which increase in number and species toward the distal end of the gut (10^{11}–10^{12} bacteria/g in the large intestine).[1,2] The gastrointestinal tract contains epithelial cells that shelter host cells from the external environment and is specially adapted to allow for colonization by beneficial commensal microorganisms to conserve host-microbial interactions and tissue homeostasis.[3,4] Perturbation of gut commensal microbial communities is known as dysbiosis and is predicted to adversely affect the immune response, leading to numerous immune-mediated inflammatory diseases.[5,6] Recent 16S rRNA and metagenomics community profiling studies of the gut microbiota have linked dysbiosis to ulcerative colitis (UC), an idiopathic relapsing disorder of the colon.[5,7–9] UC patients reportedly have reduced levels of short chain fatty acids (SCFAs), produced by intestinal microbiota to exert immunomodulatory and anti-inflammatory properties, in their feces.[10]

The incidence of UC increased in the Western countries in the latter half of the 20th century and is now rising rapidly worldwide.[11] However, the disease pathogenesis is not fully understood, despite recent advances in culture-independent sequencing and bioinformatics analyses.[12,13] Patterns of gut dysbiosis are inconsistent among published studies, partly due to differences in the specimen type (stool versus biopsy) or analytical methods used, and some patients suffer from the refractory disease.[5,7] The microbiota composition in UC patients has often been analyzed using invasive methods such as tissue biopsies, as part of a diagnostic colonoscopy.[7,8] A non-invasive method, such as an *in vitro* model that mimics the human colonic microbiota, is desirable for developing treatment strategies explicitly targeting the colonic microbiota in UC patients. *In vitro* models have been evaluated to assess microbial processes in the colon of UC patients.[14,15] However, these models do not reflect the complex microbial diversity in UC fecal donors, as shifts in microbial diversity occur during successive culture in multi-compartment reactors.[16] We,

therefore, developed an *in vitro* human colonic model using the batch fermentation system [named as the Kobe University Human Intestinal Microbiota Model (KUHIMM)], which simulates the bacterial proportions and diversity of fecal samples from healthy humans as confirmed by next-generation sequencing (NGS) analysis.[17,18] The anoxic condition in the KUHIMM was carefully constructed using the defined medium for the growth of anaerobes and constant flushing with anaerobic gas.[17,18] This model also simulated production of SCFAs in fecal donors, enabling evaluation of the effect of prebiotic oligosaccharides and dietary fibers on the colonic microbiota.[17,18] Thus, the KUHIMM fermentation system is a simple, quick, and non-invasive method useful for studying the condition of the colonic microbiota.

Current general strategies for treating UC involve induction of disease remission by treatment with 5-aminosalicylic acids (5-ASA) and/or corticosteroids in mild to moderate conditions and maintenance of remission with 5-ASA and/or thiopurines, and biological drugs (anti-tumor necrosis factor-α or vedolizumab) and cyclosporine are used for more severe conditions.[19] However, manipulating gut microbial composition with probiotics, which are live microorganisms, is also a promising and beneficial strategy for supporting the above treatments.[20–22] Clinical studies on intervention in the intestinal environment, such as fecal microbiota transplantation, successfully induced remission in active UC patients.[23,24] *Clostridium butyricum* is a probiotic strain and is known to be a butyrate producer.[25,26] Reportedly, oral administration of *C. butyricum* MIYAIRI in patients with UC who have undergone total proctocolectomy with ileal pouch-anal anastomosis prevented the development of pouchitis,[27] and the production of butyrate, propionate, and acetate was increased in the cecum of a rat model of colitis.[28] In addition, *C. butyricum* TO-A was effective for maintaining remission in quiescent UC.[29] However, the effect of administration of *C. butyricum* on colonic microbial composition and butyrate

production in UC patients is unknown. This effect can be investigated using the KUHIMM system.

The present study aimed to construct a human colonic microbiota model of UC. NGS was used for in-depth characterization of disease-associated microbiota in the *in vitro* model and fecal samples. Production of metabolites such as SCFAs by colonic microbiota was compared between UC patients and healthy subjects (HS, as control), to examine the usability of the KUHIMM system to reveal colonic metabolic conditions. We further verified the effect of *C. butyricum* MIYAIRI to restore butyrogenesis in the KUHIMM of UC patients.

2. Experimental Section

2.1. Sample Collection from Human Volunteers

Fecal samples were obtained from 13 UC patients and 13 HS who had not been treated with antibiotics for more than 2 months. Written informed consent was obtained from all participants. Demographic data and UC characteristics (disease activity, location, and medical treatment) are presented in Table S1 (Supporting Information). Disease activity was determined using the Mayo clinic score and partial Mayo score. The endoscopic Mayo clinic score was determined for patients who had undergone endoscopy within 1 month before fecal sampling (n = 11). The partial Mayo clinic score was determined at the time of fecal sampling (n = 13). Additionally, fecal samples were obtained from 14 UC patients to test the administration of *C. butyricum* MIYAIRI. After collection, fecal samples were immediately stored in an anaerobic culture swab (212550 BD BBL Culture Swab; BD Biosciences, Franklin Lakes, NJ, USA) and sent to the laboratory. The study was performed in accordance with the principles of the Declaration of Helsinki and the guidelines of our institution and approved by the institutional ethics review board at Kobe University (research code; 1902, approval date; May 10, 2016). All methods used in this study were

134 performed in accordance with approved guidelines by the Medical Ethics Committee at
135 Kobe University.
136
137 **2.2. Operation of the KUHIMM**
138 The batch fermentation system simulating the human colic microbiota used a multi-
139 channel fermenter (Bio Jr.8; ABLE, Tokyo, Japan) as described previously with some
140 modifications.[17] The system consisted of eight parallel and independent vessels. Each
141 vessel contained 100 mL of autoclaved (115 °C for 15 min) Gifu anaerobic medium (GAM
142 [Code 05422]; Nissui Pharmaceutical Co., Tokyo, Japan), with the initial pH adjusted to 6.5.
143 Anoxic conditions of vessels were constructed by purging with a mixture of N_2 and CO_2
144 (80:20) gas (15 mL/min) that was filter-sterilized through a 0.2-μm
145 polytetrafluoroethylene membrane (Pall Corporation, Port Washington, NY, USA) at 37 °C
146 for 1 h prior to fermentation. To prepare the inoculum, each fecal sample was suspended
147 in 2 mL of 0.1 M phosphate buffer (pH 6.5, consisting of a 2:1 mixture of 0.1 M NaH_2PO_4
148 and 0.1 M Na_2HPO_4) supplemented with 1% L-ascorbic acid (Wako Pure Chemical
149 Industries, Osaka, Japan). Fermentation was initiated by inoculation of each medium-
150 containing vessel with 100 μL of the above-mentioned fecal suspension. During
151 fermentation, the medium was continuously purged with a filter-sterilized gas mixture.
152 Aliquots of fermentation cultures were sampled through the side projection of the vessel.
153 Fecal samples and fermentation cultures were stored at -20°C until use.
154
155 **2.3. Preparation of *C. butyricum* MIYAIRI**
156 MIYARISAN tablets were purchased from Miyarisan Pharmaceutical Co., Ltd. (Tokyo,
157 Japan) and contained 30 mg of *C. butyricum* MIYAIRI. Precultivation was conducted at
158 37 °C in 50 mL serum vials containing 20 mL of Gifu anaerobic medium, which was flushed
159 with N_2 and CO_2 (80:20) gas and subsequently autoclaved.

160

161 **2.4. Extraction of Microbial DNA**

162 Microbial DNA was extracted from suspended feces and fermentation cultures using 0.1-
163 mm glass beads, TE (10 mM Tris-HCl, 1 mM EDTA)-saturated phenol, and sodium dodecyl
164 sulfate, as described previously.[17] Purified DNA was stored at -20°C until use.

165

166 **2.5. 16S rRNA Gene Sequencing**

167 Genomic DNA was subjected to amplification of the V3-V4 region of bacterial 16S rRNA
168 genes using the primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21.[30] Illumina
169 adapter overhang nucleotide sequences were added to the gene-specific sequences
170 (Illumina, San Diego, CA, USA). PCR was performed according to the manufacturer's
171 instructions. Confirmed amplicons were purified with AMPure XP DNA purification beads
172 (Beckman Coulter, Brea, CA, USA) and eluted in 25 μL of 10 mM Tris (pH 8.5). Amplicons
173 were quantified on Agilent Bioanalyzer 2100 DNA 1000 chips (Agilent Technologies, Santa
174 Clara, CA, USA) and pooled in equimolar concentrations (5 nM). The pooled 16S rRNA
175 gene products [along with an internal control (PhiX control V3; Illumina)] were subjected
176 to paired-end sequencing using a MiSeq sequencer (Illumina) with a 600-cycle MiSeq
177 reagent kit (Illumina). PhiX sequences were removed, and paired-end reads with Q scores
178 ≥20 were joined using QIIME version 1.9.1 software.[31] The UCLUST algorithm was used
179 to cluster filtered sequences into operational taxonomic units (OTUs) based on a 97%
180 similarity threshold.[32] Chimeric sequences were identified and excluded from the library
181 using USEARCH.[33] Paired-end reads were taxonomically classified via the Greengenes
182 taxonomic database using the Ribosomal Database Project Classifier.[33] OTUs were used
183 for diversity estimation by the Shannon-Wiener index and Simpson index and richness
184 estimation by Chao 1. Principal coordinate analysis (PCoA) was conducted using OTU
185 information from each sample and calculated based on unweighted UniFrac distances.

2.6. Real-time PCR Analysis

Real-time PCR was performed using a TP700 Thermal Cycler Dice Real Time System Lite (Takara Bio, Shiga, Japan). Amplification with a primer set targeting all eubacteria was carried out as described previously.[17]

2.7. SCFA Identification

Concentrations of SCFAs, such as acetate, propionate, and butyrate, were measured using a high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) equipped with an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA, USA) and RID-10A refractive index detector (Shimadzu).[17]

2.8. Bioinformatics and Statistical Analysis

The Shannon-Wiener diversity index, Simpson index, and Chao 1 were calculated using the QIIME software package.[31] The nonparametric Kruskal-Wallis test was used to identify significant differences in OTU numbers, Shannon-Wiener diversity index, Simpson index, Chao 1, bacterial 16S rRNA genes, and SCFA concentrations.[34] Differences in the microbial community composition, described in unweighted UniFrac distances, were tested by multivariate analysis of variance (MANOVA). Values of $P < 0.05$ were considered significant.

2.9. Accession Numbers

All raw sequence data generated in this study have been deposited in MG-RAST as “Single Batch Fermentation System Simulating Human Colonic Microbiota_Ulcerative colitis” under the accession numbers mgm4742805.3–mgm4742852.3.

3. Results

3.1. Reproduction of Numbers and Diversity of Fecal Bacterial Species in Model Culture System

Previously, the KUHIMM, *in vitro* human colonic microbiota model produced bacterial species numbers and diversity roughly equivalent to those in fecal samples from HS.[18] Initially, the stability of the gut microbiota from 4 UC patients was monitored in the cultures of KUHIMM until 48 h after inoculation of fecal donors (Figure S1, Supporting Information). Eubacterial copy numbers and OTU numbers obtained by bacterial 16S rRNA gene sequence analysis using NGS reached a plateau after 24 h of fermentation. Microbial compositions were similar after 24, 30, and 48 h of fermentation. Thus, we chose 30 h as the optimal fermentation time.

The reproducibility of bacterial species' numbers and diversity in UC fecal donors (n=13) was confirmed in the KUHIMM system and compared to those in HS (n=13) after 30 h of fermentation. Eubacterial copy numbers at 30 h reached 4.88–12.2 × 10^{10} and 3.75–11.6 × 10^{10} copies/mL in KUHIMM using fecal samples from HS and UC patients, respectively (starting from 4.49–56.4 × 10^{6} and 1.47–15.0 × 10^{6} copies/mL, respectively). Bacterial 16S rRNA gene sequence analysis of fecal samples and fermentation cultures in the KUHIMM was performed, which yielded 8,008,313 quality reads (mean, 154,006 reads/sample) (Table S2, Supporting Information). The number of OTUs approximately describes the number of species. OTU numbers in fecal samples of UC patients were maintained in corresponding fermentation cultures of the *in vitro* model (P = 0.12, Kruskal-Wallis), as observed for HS (P = 0.41, Kruskal-Wallis; Figure 1A). Species diversity was evaluated using both the Shannon-Wiener index (sensitive to the number of species) and Simpson index (sensitive to the relative abundance of species).[35] Species richness was evaluated by Chao 1. Shannon-Wiener index, Simpson index, and Chao 1 scores in fecal samples of UC patients were not significantly different from those in corresponding

fermentation cultures (P = 0.47, 0.38, and 0.44, respectively, Kruskal-Wallis), as observed for HS (P = 0.20, 0.20, and 0.47, respectively, Kruskal-Wallis) (Figures 1B, 1C and 1D). Species diversity and richness in UC fecal samples were maintained in corresponding fermentation cultures in the KUHIMM.

OTU numbers in fecal samples did not significantly differ between HS and UC patients (P = 0.50, Kruskal-Wallis; Figure 1A), and this tendency was reproduced in corresponding fermentation cultures between HS and UC patients (P = 0.36, Kruskal-Wallis). Additionally, no significant differences in the Shannon-Wiener index, Simpson index, and Chao 1 scores of fecal samples were observed between HS and UC patients (P = 0.10, 0.20, and 0.28, respectively, Kruskal-Wallis), and these tendencies were reproduced in corresponding fermentation cultures between HS and UC patients (P = 0.07, 0.22, and 0.92, respectively, Kruskal-Wallis) (Figures 1B, 1C and 1D). Species diversity and richness were similar between HS and UC patients for both fecal samples and corresponding fermentation cultures of the KUHIMM.

3.2. Altered Microbiota Compositions in Fecal Samples and Fermentation Cultures of UC Patients

Comparison of the principal coordinate plots of the unweighted UniFrac distance revealed that fecal samples of UC patients approximately formed different clusters from those of HS (Figure 1E). This result suggests that microbiota compositions in fecal samples from UC patients differed from those from HS (P = 0.001, MANOVA). These differences in microbiota composition between HS and UC patients were also observed in corresponding fermentation cultures in *in vitro* colonic microbiota models (P = 0.001, MANOVA).

Most sequences in all samples were associated with six bacterial phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, and *Verrucomicrobia* (Figure 2A). Microbiota compositions of fecal samples and corresponding

fermentation cultures of HS and UC patients were also examined at the family level. Interestingly, the proportions of species in the family *Lachnospiraceae* were significantly decreased in fecal samples of UC patients compared to those in fecal samples of HS (P = 0.001, Kruskal-Wallis). Similarly, proportions of family *Lachnospiraceae* were significantly decreased in the corresponding fermentation cultures of UC patients compared to those in corresponding cultures of HS (P = 0.001, Kruskal-Wallis) (Figure S2, Supporting Information). Significant increases in the families *Bifidobacteriaceae* (P = 0.024, Kruskal-Wallis), *Enterococcaceae* (P = 0.008, Kruskal-Wallis) and *Enterobacteriaceae* (P = 0.004, Kruskal-Wallis) in fecal samples of UC patients compared to those in fecal samples of HS were observed, and the mean relative abundances of these families were generally increased in the corresponding fermentation cultures, although significant differences were not observed (P = 0.190, 0.094, and 0.200, respectively, Kruskal-Wallis).

The family *Lachnospiraceae* contained uncultured *Lachnospiraceae* spp. and genera *Blautia*, *Coprococcus*, *Dorea*, *Lachnospira*, and *Roseburia* (Table 1), all of which belong to *Clostridium* cluster XIVa.[36] Species related to uncultured *Lachnospiraceae* and belonging to the genera *Blautia*, *Coprococcus*, and *Roseburia* were significantly reduced in fecal samples of UC patients compared to those in fecal samples of HS (P = 0.0019, 0.0019, 0.0103, and 0.0137, respectively, Kruskal-Wallis). Bacterial species related to uncultured *Lachnospiraceae* and belonging to the genus *Coprococcus* were also decreased in corresponding fermentation cultures of UC patients compared to those in corresponding cultures of HS (P = 0.0044 and 0.0167, respectively, Kruskal-Wallis). Mean relative abundances of bacterial species belonging to the genera *Blautia* and *Roseburia* were generally decreased in corresponding fermentation cultures of UC patients compared to those in corresponding cultures of HS, although significant differences were not observed (P = 0.0685 and 0.0540, respectively, Kruskal-Wallis).

3.3. Decreased Butyrogenesis in Model Culture Systems of UC Patients

SCFAs are the primary end-products of fermentation of dietary fibers and play a role as crucial signaling molecules between the gut microbiota and host health.[37] SCFA production was compared among KUHIMM-simulating colonic microbiotas of UC patients (n = 13) and HS (n = 13) after 30 h of fermentation. Acetate was the primary product, followed by propionate and butyrate, in corresponding fermentation cultures of both HS and UC patients. Lactate and succinate levels were scarce. Interestingly, butyrate production was decreased in the corresponding fermentation cultures of UC patients compared to those in corresponding cultures of HS ($P < 0.01$, Kruskal-Wallis) (Figure 2B). The production of acetate and propionate was similar between HS and UC patients (P = 0.22 and 0.36, respectively, Kruskal-Wallis) (Figures 2C and 2D). SCFA production (sum of lactate, succinate, acetate, propionate, and butyrate) was decreased in the corresponding fermentation cultures of UC patients compared to in corresponding cultures of HS (P = 0.02, Kruskal-Wallis) (Figure 2E).

Loss of organisms from the *Lachnospiraceae* family correlated with decreased butyrate production in the KUHIMM (Figure 3A). Disease activity indices, i.e., partial Mayo score or Mayo score, did not show proportional relationships with the relative abundance of family *Lachnospiraceae* in both corresponding fermentation cultures and fecal samples of UC patients (Figures 3B and 3C, and Figure S3, Supporting Information). Accordingly, butyrate production in the KUHIMM did not show proportional relationships with the disease activity index (Figure S4, Supporting Information).

3.4. Recovery of Butyrogenesis by Administration of *C. butyricum* MIYAIRI in Model Culture System of UC Patients

The spore-forming bacteria *C. butyricum* MIYAIRI can survive in the stomach and tolerate bile salts.[23] We investigated whether administration of *C. butyricum* MIYAIRI can increase

316 butyrogenesis. Different doses of *C. butyricum* MIYAIRI (5×10^6, 5×10^5, or 5×10^4
317 cells/mL) were added into the KUHIMM for each fecal donor of UC patients (n = 14). No
318 addition of cells was used as a control. Bacterial 16S rRNA gene sequence analysis was
319 performed for the fecal samples of patients and the corresponding fermentation cultures
320 in the KUHIMM. *Clostridium butyricum* was scarcely detected in UC feces and the relative
321 abundance of *C. butyricum* was <0.3% among the total bacteria in most (12) cultures of the
322 KUHIMM after 30 h of fermentation (Figure 4A). By adding 5×10^6, 5×10^5, or 5×10^4
323 cells/mL of *C. butyricum* MIYAIRI, the relative abundance of *C. butyricum* increased to 20.4
324 ± 10.4%, 8.7 ± 6.2%, or 6.3 ± 10.4%, respectively, after a 30-h fermentation (Figure 4A).
325 Addition of *C. butyricum* MIYAIRI significantly increased butyrate production in the
326 KUHIMM compared to no addition of *C. butyricum* MIYAIRI (P<0.01, Kruskal-Wallis)
327 (Figure 4B). Addition of *C. butyricum* MIYAIRI significantly decreased acetate production
328 in the KUHIMM compared to no addition of *C. butyricum* MIYAIRI ($P < 0.05$, Kruskal-
329 Wallis) (Figure 4C). High occupation of *C. butyricum* (20.4%) decreased propionate
330 production ($P < 0.05$, Kruskal-Wallis) (Figure 4D). Lactate and succinate levels were
331 scarce in most cultures (Figure S5, Supporting Information). As a result, SCFA
332 concentrations were decreased by the addition of 5×10^6 and 5×10^4 cells/mL of *C.*
333 *butyricum* MIYAIRI compared to no addition (Figure 4E). Addition of 5×10^4 cells/mL of *C.*
334 *butyricum* MIYAIRI did not affect the microbiota composition in the KUHIMM (Figures S6
335 and S7, Supporting Information). Thus, 6% *C. butyricum* in the human colonic microbiota
336 of UC patients may be sufficient to increase butyrogenesis without changing the colonic
337 microbiota composition.
338
339 **4. Discussion**
340 Microbiota diversity in UC patients, as evaluated by Shannon-Wiener index, Simpson index,
341 and Chao 1, was similar to that in HS in both fecal samples and corresponding

fermentation cultures. As described previously, no significant difference was observed in the α diversity index of luminal samples from UC patients compared to those from HS.[7] This system represents the first *in vitro* human colonic microbiota model that is able to maintain the bacterial species number and diversity observed in UC patient feces. Additionally, we successfully reproduced human colonic microbial communities from UC patients that differed from those in HS using our *in vitro* model, KUHIMM, consistent with a previous research showing differences in microbiomes of fecal samples from UC patients compared to those from HS.[38] Thus, the KUHIMM of UC reproduced a significant decrease in butyrate production, unlike the other simulator of the human intestinal microbial ecosystem (consisting of multiple vessels) that did not show a significant decrease in butyrate production using fecal samples from UC patients.[15] Although butyrate is responsible for essential physiological and pathophysiological functions,[10,39] evaluation of butyrate production in the human body is generally challenging; interestingly, KUHIMM was able to detect impaired butyrogenesis in UC patients, and thus, may be useful for evaluating butyrate production.

Franke et al. reported a decreased abundance of the family *Lachnospiraceae* in the colon of UC patients compared to that in the colon of healthy controls.[9] Our results also revealed a decreased abundance of the family *Lachnospiraceae* in fecal samples from UC patients. Reduced abundance of the family *Lachnospiraceae* has been associated with severe inflammation in a murine model of colitis, and administration of *Lachnospiraceae* bacteria suppressed colitis.[40] Notably, KUHIMM reproduced the decreased abundance of this family in the cultures inoculating UC fecal samples. Bacterial members of the family *Lachnospiraceae* are known to be the predominant constituents of mammalian gastrointestinal tract microbiomes, particularly in ruminants and humans, and have been implicated in butyric acid production.[41] Butyrate is an excellent source of energy for host epithelial cells.[42] Furthermore, butyrate exerts an anti-inflammatory effect that results

368 from the generation of peripheral regulatory T cells and can block activation of pathogen-
369 induced nuclear factor kappa B.[43–46] Additionally, butyrate inhibits the growth of
370 cancerous colonic cells,[47,48] and lower butyrate concentrations in the mouse intestinal
371 lumen reportedly increased epithelial oxygenation to allow for the growth of aerobic
372 *Salmonella*, which is a causative pathogen of gastroenteritis.[49] These considerations
373 suggest that low butyrate production, as observed in the KUHIMM, triggers an
374 inflammatory state in UC patients. Recent *in vitro* studies suggested that *Clostridium*
375 cluster XIVa groups within the family *Lachnospiraceae* are enriched in the anatomical
376 barrier, i.e., the intestinal mucosa, and interact with other microorganisms via cross-
377 feeding.[36,50] Decreases in *Clostridium* cluster XIVa groups in the lumen, as suggested by
378 the KUHIMM, may exacerbate microbial ecology in the mucous layer.

379 The results showing no correlation between disease activity and the relative
380 abundance of the family *Lachnospiraceae* in fecal samples and KUHIMM cultures suggest
381 that the family *Lachnospiraceae* may be less abundant at any phase, even during the
382 remission state of UC activity in the patient's intestine. As UC is characterized by a
383 repetitive disease phase of exacerbation and remission, the decreased abundance of the
384 family *Lachnospiraceae* and the resulting low butyrogenesis may play a role in triggering
385 the recurrence of UC. Increasing butyrate-producing organisms in the colon of UC patients
386 may be a critical factor in sustaining the remission phase. The use of pre- and probiotics
387 that act on the microbial composition has been evaluated in recent decades.[51] Our simple
388 *in vitro* human colonic microbiota model, KUHIMM, can be utilized for high-throughput
389 evaluation of the role of pre- and probiotics in commensal bacteria and metabolite
390 production in UC patients. Spore-forming *C. butyricum* has been used safely in Japanese
391 clinics for over 40 years.[27] It is important to investigate whether oral administration of *C.*
392 *butyricum* MIYAIRI is sufficient to reach approximately 6% of the human colonic

393 microbiota to restore the production of butyrate and to affect the human colonic
394 microbiota to maintain UC remission in future clinical studies.

395 Our KUHIMM showed the tendency to reproduce the increase in the numbers of
396 bacteria belonging to the families *Bifidobacteriaceae*, *Enterococcaceae*, or
397 *Enterobacteriaceae*, which were previously observed in UC patients.[52–54] However,
398 KUHIMM did not inhibit the growth of the family *Peptostreptococcaceae*. Some differences
399 in the microbiota species composition between fecal samples and corresponding
400 fermentation cultures were observed, which may be a limitation of this study (Figure 2).
401 Reportedly, antimicrobial peptides and immunoglobulin A produced by the intestinal
402 epithelium and plasma cells in the lamina propria, respectively, are secreted into the
403 intestinal lumen to control the spatial segregation and composition of the gut flora.[4,55,56]
404 In the KUHIMM, host factors responsible for flora regulation were missing during a 30-h
405 culture. Adding these factors into the KUHIMM may lead to more accurate and successful
406 reproduction of the results for certain bacterial members.

407 In conclusion, our new culture system confirmed the reduced levels of the family
408 *Lachnospiraceae* and reduced butyrate level in UC patients. Our culture system can be
409 used as a model to evaluate the intestinal environment of UC patients and effects of
410 probiotic strains.

411

412

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Conflict of Interest

The authors declare no financial or commercial conflict of interest.

References

[1] R. Sender, S. Fuchs, R. Milo, *PloS Biol.* **2016**, *14*, e1002533.

[2] A.M. O'Hara, F. Shanahan, *EMBO Rep.* **2006**, 7, 688.

[3] L.W. Peterson, D. Artis, *Nat. Rev. Immunol.* **2014**, *14*, 141.

[4] L.V. Hooper, A.J. Macpherson, *Nat. Rev. Immunol.* **2010**, *10*, 159.

[5] J.D. Forbes, G. Van Domselaar, C.N. Bernstein, *Front. Microbiol.* **2016**, *11*, 1081.

[6] D. Gevers, S. Kugathasan, L.A. Denson, Y. Vázquez-Baeza, W. Van Treuren, B. Ren, E. Schwager, D. Knights, S.J. Song, M. Yassour, X.C. Morgan, *Cell Host Microbe* **2014**, *15*, 382.

[7] A. Lavelle, G. Lennon, O. O'sullivan, N. Docherty, A. Balfe, A. Maguire, H.E. Mulcahy, G. Doherty, D. O'donoghue, J. Hyland, R.P. Ross, *Gut.* **2015**, *64*, 1553.

[8] X.C. Morgan, T.L. Tickle, H. Sokol, D. Gevers, K.L. Devaney, D.V. Ward, J.A. Reyes, S.A. Shah, N. LeLeiko, S.B. Snapper, A. Bousvaros, *Genome Biol.* **2012**, *13*, R79.

[9] D.N. Frank, A.L. Amand, R.A. Feldman, E.C. Boedeker, N. Harpaz, N.R. Pace, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 13780.

[10] K. Machiels, M. Joossens, J. Sabino, V. De Preter, I. Arijs, V. Eeckhaut, V. Ballet, K. Claes, F. van Immerseel, K. Verbeke, M. Ferrante, *Gut* **2014**, *63*, 1275.

[11] Y. Hirata, S. Ihara, K. Koike, *Pharmacol. Res.* **2016**, *113*, 574.

[12] S.C. Ng, H.Y. Shi, N. Hamidi, F.E. Underwood, W. Tang, E.L. Benchimol, R. Panaccione, S. Ghosh, J.C. Wu, F.K. Chan, J. J. Sung, *Lancet* **2018**, *390*, 2769.

[13] N.A Molodecky, S. Soon, D.M. Rabi, W.A. Ghali, M. Ferris, G. Chernoff, E.I. Benchimol, R. Panaccione, S. Ghosh, H.W. Barkema, G.G. Kaplan, *Gastroenterology* **2012**, *142*, 46

[14] L.K. Vigsnaes, P. Van den Abbeele, K. Sulek, H.L. Frandsen, C. Steenholdt, J. Brynskov, J. Vermeiren, T. Van De Wiele, T. Licht, *Sci. Rep.* **2013**, *3*, 1110.

[15] J. Vermeiren, P. Van den Abbeele, D. Laukens, L.K. Vigsnæs, M. De Vos, N. Boon, T. Van de Wiele, *FEMS Microbiol. Ecol.* **2012**, *79*, 685.

[16] M. Rajilić-Stojanović, A. Maathuis, H.G. Heilig, K. Venema, W. M. de Vos, H. Smidt, *Microbiology* **2010**, *156*, 3270.

[17] R. Takagi, K. Sasaki, D. Sasaki, I. Fukuda, K. Tanaka, K.I. Yoshida, A. Kondo, R. Osawa, *PLoS One* **2016**, *11*, e0160533.

[18] D. Sasaki, K. Sasaki, N. Ikuta, T. Yasuda, I. Fukuda, A. Kondo, R. Osawa, *Sci. Rep.* **2018**, *11*, 435.

[19] R. Ungaro, S. Mehandru, P.B. Allen, L. Peyrun-Biroulet, J.F. Colombel, *Lancet* **2017**, *389*, 1756.

[20] L. A. Derikx, L.A. Dieleman, F. Hoentjen, *Best Pract. Res. Clin. Gastroenterol.* **2016**, *30*, 55.

[21] W. Kruis, P. Frič, J. Pokrotnieks, M. Lukáš, B. Fixa, M. Kaščák, M.A. Kamm, J. Weismueller, C. Beglinger, M. Stolte, C. Wolff, *Gut* **2004**, *53*, 1617.

[22] B.J. Rembacken, A.M. Snelling, P.M. Hawkey, D.M. Chalmers, A.T. Axon, *Lancet* **1999**, *354*, 635.

[23] S. Paramsothy, M.A. Kamm, N.O. Kaakoush, A.J. Walsh, J. van den Bogaerde, D. Samuel , R.W. Leong, S. Connor, W. Ng, R. Paramsothy, W. Xuan, *Lancet.* **2017**, *389*, 1218.

[24] P. Moayyedi, M.G. Surette, P.T. Kim, J. Libertucci, M. Wolfe, C. Onischi, D. Armstrong, J.K. Marshall, Z. Kassam, W. Reinisch, C.H. Lee, *Gastroenterology* **2015**, *49*, 102

[25] J. Zhang, X. Chen, P. Liu, J. Zhao, J. Sun, W. Guan, L. J. Johnston, C.L. Levesque, P. Fan, T. He, G. Zhang, *J. Agric. Food Chem.* **2018**, *66*, 5157.

[26] M. Cai, L. Zeng, L. J. Li, L. H. Mo, R. D. Xie, B. S. Feng, P. Y. Zheng, Z.G. Liu, Z. J. Liu, P.C. Yang, *Allergy Asthma Clin. Immunol.* **2016,** *12*, 37.

[27] A. Yasueda, T. Mizushima, R. Nezu, R. Sumi, M. Tanaka, J. Nishimura, Y. Kai, M. Hirota, H. Osawa, K. Nakajima, M. Mori, *Surg. Today* **2016**, *46*, 939.

[28] T. Okamoto, M. Sasaki, T. Tsujikawa, Y. Fujiyama, T. Bamba, M. Kusunoki, *J Gastroenterol.* **2000**, *35*, 341.

[29] Y. Yoshimatsu, A. Yamada, R. Furukawa, K. Sono, A. Osamura, K. Nakamura, H. Aoki, Y. Tsuda, N. Hosoe, N. Takada, Y. Suzuki, *World J. Gastroenterol.* **2015**, *21*, 5985.

[30] A. Klindworth, E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, F.O. Glöckner, *Nucleic Acids Res.* **2013**, *41*, e1.

[31] J.G Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Pena, J.K. Goodrich, J. I. Gordon, G.A. Huttley, *Nat. Methods* **2010**, 7, 335.

[32] R.C. Edgar. *Bioinformatics* **2010**, *26*, 2460.

[33] Q. Wang, G.M. Garrity, J. M. Tiedje, J.R. Cole, *Appl. Environ. Microbiol.* **2007**, *73*, 5261

[34] W. Kruskal, W. Wallis, *J. Am. Statist. Assoc.* **1952**, *47*, 583.

[35] K.V.A Johnson, P.W.J. Burnet, *Gut Microbes* **2016**, *7*, 455.

[36] L.R. Lopetuso, F. Scaldaferri, V. Petito, A. Gasbarrini, *Gut Pathog.* **2013**, *5*, 23.

[37] D.J. Morrison, T. Preston, *Gut Microbes* **2016**, *7*, 189.

[38] V. Pascal, M. Pozuelo, N. Borruel, F. Casellas, D. Campos, A. Santiago, X. Martinez, E. Varela, G. Sarrabayrouse, K. Machiels, S. Vermeire, *Gut* **2017**, *66*, 813.

[39] W. Scheppach, H. Sommer, T. Kirchner, G.M. Paganelli, P. Bartram, S. Christl, F. Richter, G. Dusel, H. Kasper, *Gastroenterology* **1992**, *103*, 51.

[40] L. Chen, J.E. Wilson, M.J. Koenigsknecht, W.C. Chou, S.A. Montgomery, A.D. Truax, W.J. Brickey, C.D. Packey, N. Maharshak, G.K. Matsushima, S. Plevy. *Nat. Immunol.* **2017**, *18*, 541.

[41] C.J. Meehan, R.G. Beiko, *Genome Biol. Evol.* **2014**, *6*, 703.

[42] S.E. Pryde, S.H. Duncan, G.L. Hold, C.S. Stewart, H.J. Flint, *FEMS Microbiol. Lett.* **2002**, *17*, 133.

[43] N. Arpaia, C. Campbell, X. Fan, S. Dikiy, J. van der Veeken, H. Liu, J.R. Cross, K. Pfeffer, P.J. Coffer, A.Y. Rudensky, *Nature* **2013***, 504*, 451.

[44] Y. Furusawa, Y. Obata, S. Fukuda, T.A. Endo, G. Nakato, D. Takahashi, Y. Nakanishi, C. Uetake, K. Kato, T. Kato, M. Takahashi, *Nature* **2013**, *504*, 451.

[45] P. Louis, G.L. Hold, H.J. Flint, *Nat. Rev. Microbiol.* **2014**, *12*, 661.

[46] H. Lührs, T. Gerke, J. Schauber, G. Dusel, R. Melcher, W. Scheppach, T. Menzel, *Int. J. Colorectal Dis.* **2001**, *16*, 195–201.

[47] K.Y. Fung, L. Cosgrove, T. Lockett, T. Head, D.L.Topping, *Br. J. Nutr.* **2012**, *108*, 820.

[48] J.M. Clarke, D.L. Topping, A.R. Bird, G.P. Young, L. Cobiac, *Carcinogenesis* **2008**, *29*, 2190.

[49] F. Rivera-Chávez, L.F. Zhang, F. Faber, C.A. Lopez, M.X. Byndloss, E.E. Olsan, G. Xu, E.M. Velazquez, C.B. Lebrilla, S.E. Winter, A.J. Bäumler. *Cell Host Microbe* **2016**, *19*, 443.

[50] P. Van den Abbeele, C. Belzer, M. Goossens, M. Kleerebezem, W.M. De Vos, O. Thas, R. De Weirdt, F.M. Kerckhof, T. Van de Wiele, *ISME J*. **2013**, *7*, 949.

[51] L.A. Derikx, L.A. Dieleman, F. Hoentjen, *Best Pract. Res. Clin. Gastroenterol.* **2016**, *30,* 55.

[52] S.A. Walujkar, D.P. Dhotre, N.P. Marathe, P.S. Lawate, R.S. Bharadwaj, Y.S. Shouche, *Gut Pathog.* **2014**, *6*, 22.

[53] M. Rajilić-Stojanović, F. Shanahan, F. Guarner, W.M. de Vos, *Inflamm. Bowel Dis.* **2013**, *19*, 481.

[54] L.K. Vigsnæs, J. Brynskov, C. Steenholdt, A. Wilcks, T.R. Licht *Benef. Microbes* **2012**, *3*, 287.

[55] S. Okai, F. Usui, S. Yokota, Y. Hori-i, M. Hasegawa, T. Nakamura, M. Kurosawa, S. Okada, K. Yamamoto, E. Nishiyama, H. Mori, *Nat. Microbiol.* **2016**, *1*, 16103.

[56] S. Vaishnava, M. Yamamoto, K.M. Severson, K.A. Ruhn, X. Yu, O. Koren, R. Ley, E.K. Wakeland, L.V. Hooper, *Science* **2011**, *334*, 255.

525 **Table 1.** Genus-level distribution of bacteria belonging to the family *Lachnospiraceae* in
526 fecal samples and corresponding fermentation cultures of healthy subjects (HS) and
527 ulcerative colitis (UC) patients.

Bacteria taxa (%) *Lachnospiraceae*	HS Feces (n = 13)	HS Culture (n = 13)	UC Feces (n = 13)	UC Culture (n = 13)
Uncultured *Lachnospiraceae* spp.	9.62 ± 6.75	9.00 ± 2.96	3.48 ± 3.68**	4.61 ± 3.46**
Blautia spp.	9.63 ± 4.46	3.90 ± 2.51	4.22 ± 4.39**	2.70 ± 3.53
Coprococcus spp.	2.72 ± 1.72	2.73 ± 2.96	1.11 ± 1.33*	0.82 ± 1.30*
Dorea spp.	0.96 ± 0.43	0.54 ± 0.56	0.87 ± 0.85	0.73 ± 1.02
Lachnospira spp.	1.78 ± 2.84	0.00 ± 0.01	0.33 ± 0.53	0.00 ± 0.01
Roseburia spp.	0.73 ± 0.87	0.05 ± 0.06	0.05 ± 0.08*	0.02 ± 0.08

528 Asterisks (*) indicate significant differences in fecal samples and corresponding
529 fermentation cultures between HS and UC patients (*P < 0.05 and **P < 0.01, Kruskal-
530 Wallis).
531 Genera of lower abundance were omitted.
532

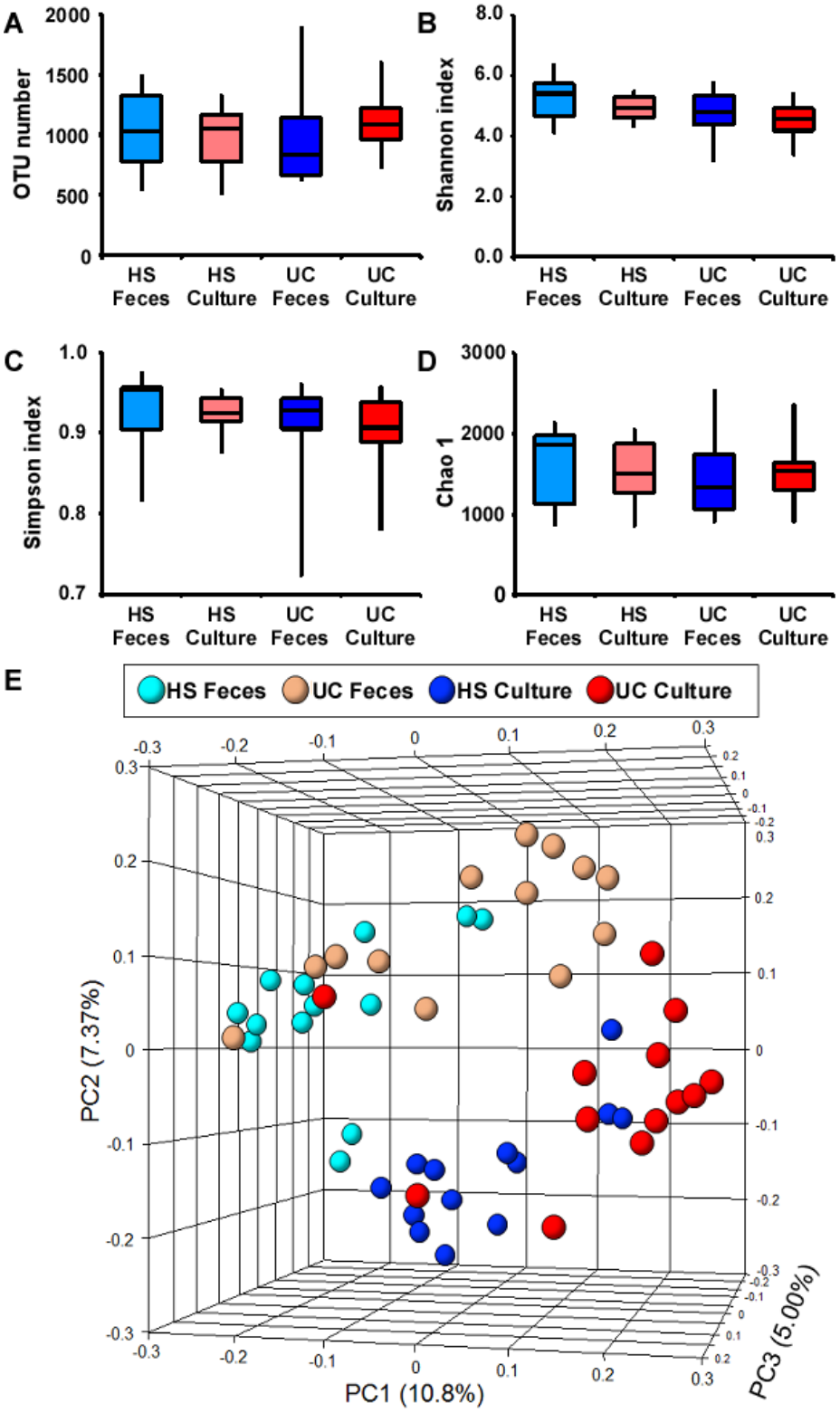
Figure legends

Figure 1. Bacterial comparisons between in fecal samples and corresponding cultures of healthy subjects (HS) and ulcerative colitis (UC) patients. (A) Operational taxonomic unit (OTU) number, (B) Shannon index, (C) Simpson index, and (D) Chao1. (E) Principal coordinate analysis (PCoA) of bacterial 16S rRNA gene data. Score plot shows data on fecal samples from healthy subjects (HS) (light blue) and UC patients (light brown) and corresponding fermentation cultures of HS (blue) and UC patients (red). Sampling was performed after 30 h of fermentation.

Figure 2. (A) Family-level compositional view of bacteria. 16S rRNA genes were evaluated in fecal samples of healthy subjects (HS) and ulcerative colitis (UC) patients and corresponding cultures after 30 h of fermentation. Families of lower abundance were included in "Others". Production of (B) butyrate, (C) acetate, (D) propionate, and (E) short-chain fatty acids (sum of lactate, succinate, acetate, propionate and butyrate). Asterisks (*) indicate significant differences between HS and UC patients (*$P < 0.05$ and **$P < 0.01$, Kruskal-Wallis). ns: not significant.

Figure 3. Relationship between family *Lachnospiraceae* and butyrate concentration or partial Mayo score. (A) Correlation between butyrate concentration (mM) and relative abundance of family *Lachnospiraceae* in *in vitro* ulcerative colitis (UC) cultures (●) and healthy subject (HS) cultures (○) after 30 h of fermentation. (B) Correlation between UC partial Mayo score and relative abundance of family *Lachnospiraceae* in *in vitro* UC cultures after 30 h of fermentation. (C) Correlation between UC partial Mayo score and relative abundance of family *Lachnospiraceae* in UC fecal samples. Solid lines and corresponding equations indicate the best-fit linear relationship.

559 **Figure 4.** Effect of addition of *Clostridium butyricum* MIYAIRI into the KUHIMM. (A) The
560 average of the relative abundance of *C. butyricum* in the fecal donors of four ulcerative
561 colitis (UC) patients and corresponding cultures adding 0, 5×10^6, 5×10^5, or 5×10^4 of *C.*
562 *butyricum* MIYAIRI. The cultures were sampled after 30 h of fermentation. Production of
563 (B) butyrate, (C) acetate, (D) propionate, and (E) short-chain fatty acids (sum of lactate,
564 succinate, acetate, propionate, and butyrate). Asterisks (*) indicate significant differences
565 between the addition of *C. butyricum* MIYAIRI and no addition (*$P < 0.05$ and **$P < 0.01$,
566 Kruskal-Wallis). ns: not significant.
567


A
OTU number
2000
1500
1000
500
0
HS Feces
HS Culture
UC Feces
UC Culture
B
Shannon index
8.0
6.0
4.0
2.0
0.0
C
Simpson index
1.0
0.9
0.8
0.7
D
Chao 1
3000
2000
1000
0
E
HS Feces
UC Feces
HS Culture
UC Culture
PC1 (10.8%)
PC2 (7.37%)
PC3 (5.00%)

568

Figure 1

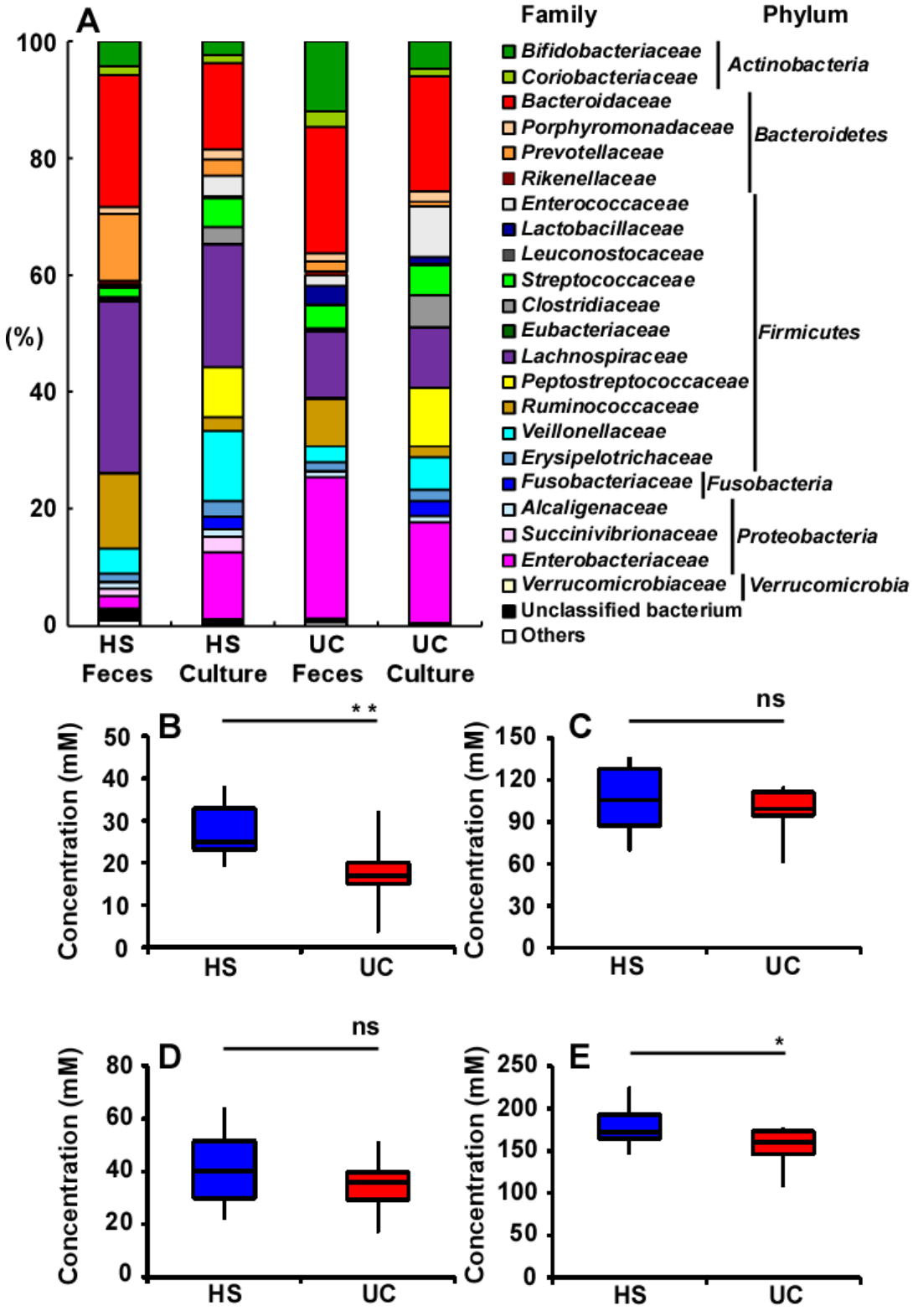
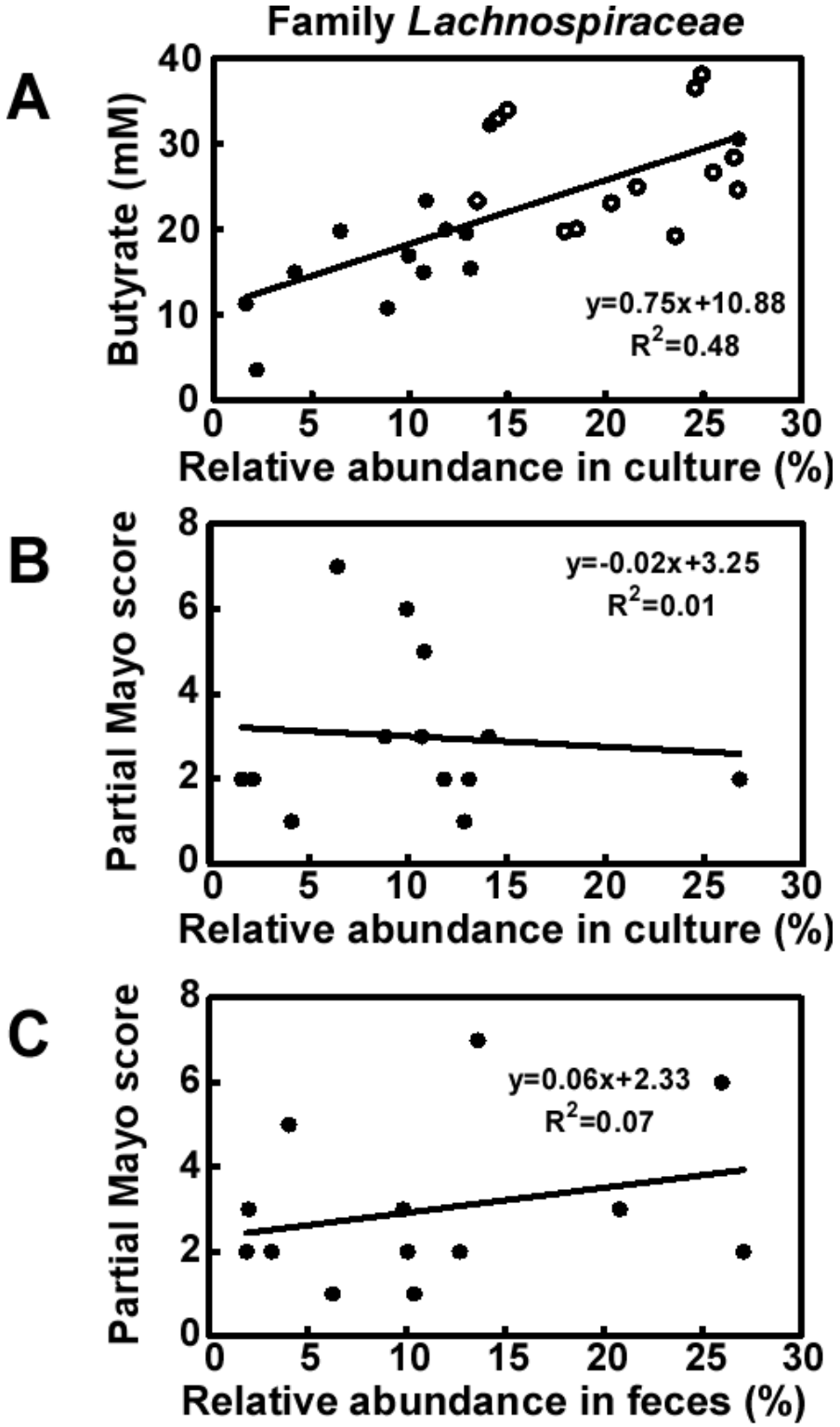
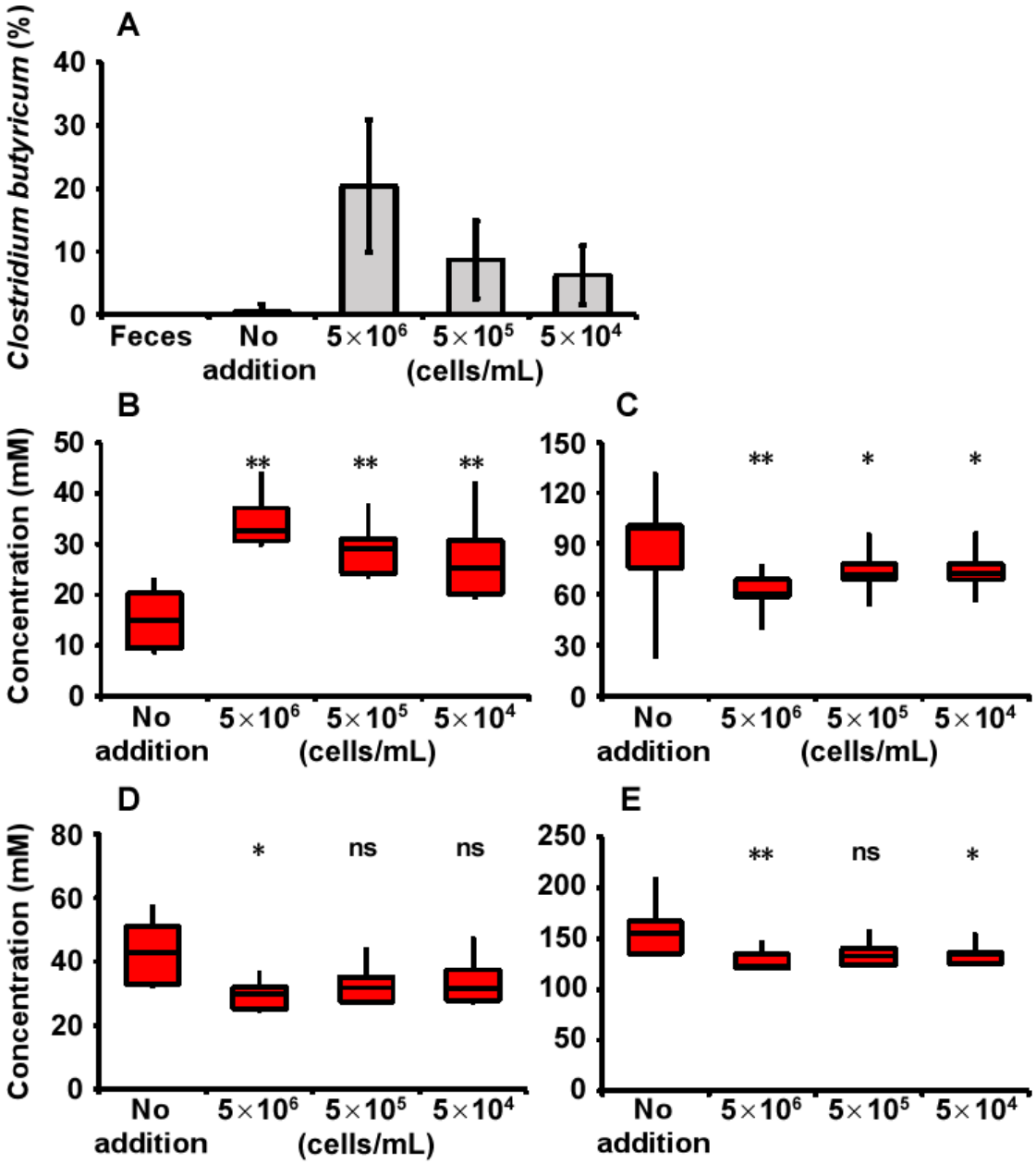

A
100
80
60
(%)
40
20
0
HS Feces
HS Culture
UC Feces
UC Culture
Family
Phylum
Bifidobacteriaceae
Coriobacteriaceae
Actinobacteria
Bacteroidaceae
Porphyromonadaceae
Prevotellaceae
Rikenellaceae
Bacteroidetes
Enterococcaceae
Lactobacillaceae
Leuconostocaceae
Streptococcaceae
Clostridiaceae
Eubacteriaceae
Lachnospiraceae
Peptostreptococcaceae
Ruminococcaceae
Veillonellaceae
Erysipelotrichaceae
Firmicutes
Fusobacteriaceae
Fusobacteria
Alcaligenaceae
Succinivibrionaceae
Enterobacteriaceae
Proteobacteria
Verrucomicrobiaceae
Verrucomicrobia
Unclassified bacterium
Others
B
**
Concentration (mM)
50
40
30
20
10
0
HS
UC
C
ns
Concentration (mM)
150
120
90
60
30
0
HS
UC
D
ns
Concentration (mM)
80
60
40
20
0
HS
UC
E
*
Concentration (mM)
250
200
150
100
50
0
HS
UC

Figure 2.



571 **Figure 3.**

572



573 **Figure 4.**

574

Supporting Information

Construction of a model culture system of human colonic microbiota to detect decreased *Lachnospiraceae* abundance and butyrogenesis in the feces of ulcerative colitis patients

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Table S1. Characteristics of study volunteers comprising healthy subjects (HS) and ulcerative colitis (UC) patients

	HS (n = 13)	UC (n = 13)
Age in years (mean ± SD)	39.5 ± 10.6	50.6 ± 18.1
Sex (F/M)	6/7	7/6
Race		
Asian	100%	100%
Mean disease duration, years (mean ± SD)	NA	7.3 ± 9.7
Disease activity		
Mayo score	NA	5.1 ± 2.7 (n = 11)
Partial Mayo score	NA	3.0 ± 1.9 (n = 13)
Disease location		
E1/E2/E3	NA	4/2/7
Medical treatment[a]		
5-ASAs	0	10
Oral corticosteroids	0	1
Purine analogues	0	6
TNF inhibitors	0	0
Other immunosuppressant	0	1

NA: Not applicable; 5-ASA: 5-aminosalicylic acid; TNF: Tumor necrosis factor.

[a]Medical treatment at the time of sample collection.

606 **Table S2. Reads of bacterial 16S rRNA gene sequencing in healthy subjects (HS) and**

607 **ulcerative colitis (UC) patients**

	HS Feces (n = 13)	HS Culture (n = 13)	UC Feces (n = 13)	UC Culture (n = 13)
Reads	**125,807 ± 56,942**	**98,849 ± 37,682**	**193,397 ± 68,797**	**197,971 ± 62,355**

608

609

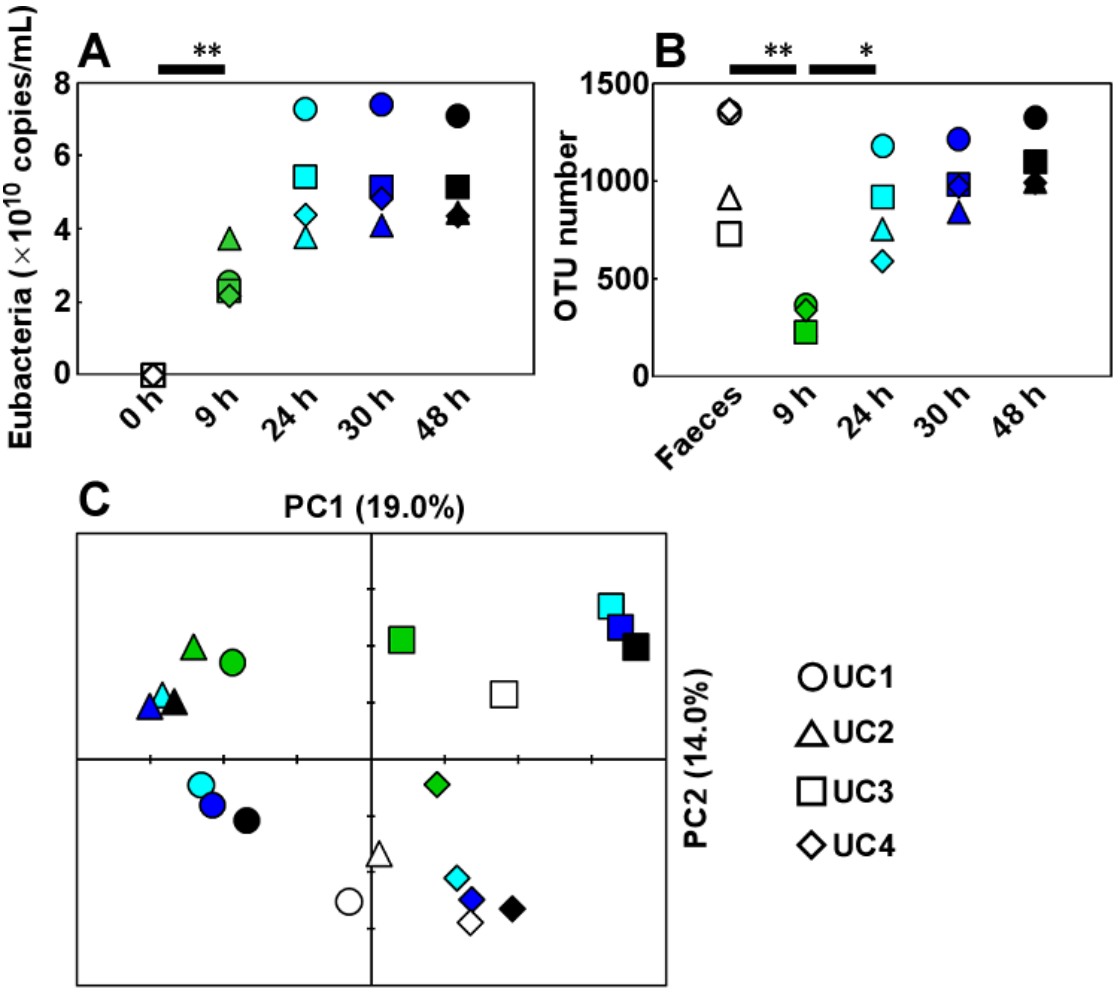


Figure S1.

Figure S1. Microbial succession in the KUHIMM of 4 UC patients. (A) Eubacterial copy numbers in the KUHIMM at 0 (white), 9 (green), 24 (light blue), 30 (blue), and 48 (black) h after the initiation of fermentation. Asterisks (*) indicate significant difference (**P < 0.01, paired t-test). (B) Observed operational taxonomic unit (OTU) number in human fecal samples (white) and the corresponding cultures at 9, 24, 30, and 48 h after the initiation of fermentation. *P < 0.05, **P < 0.01, paired t-test. (C) Principal coordinate analysis (PCoA) of 16S metagenomic data of bacterial species in human fecal samples and the corresponding cultures at 9, 24, 30, and 48 h after the initiation of fermentation.

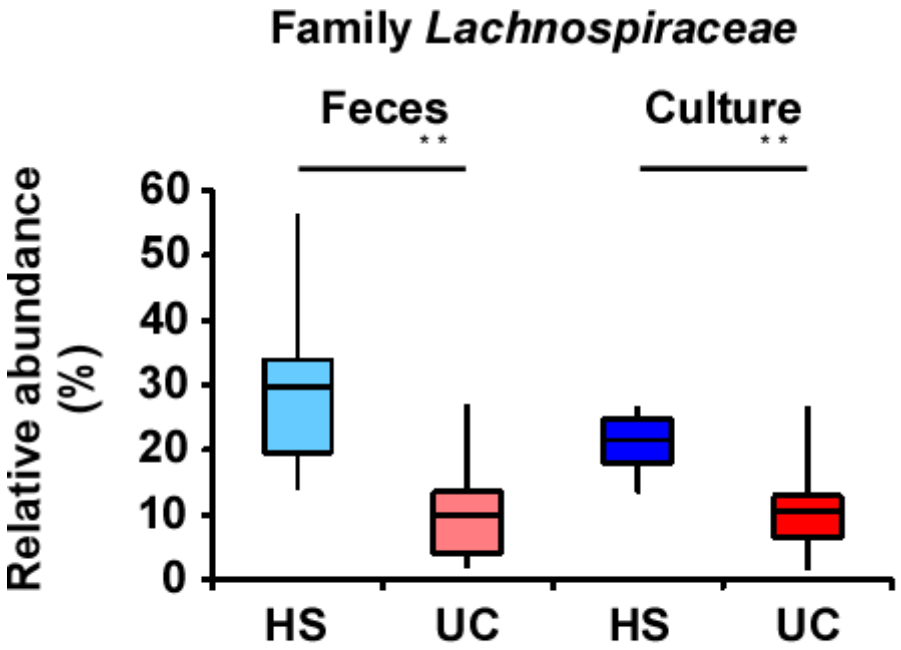
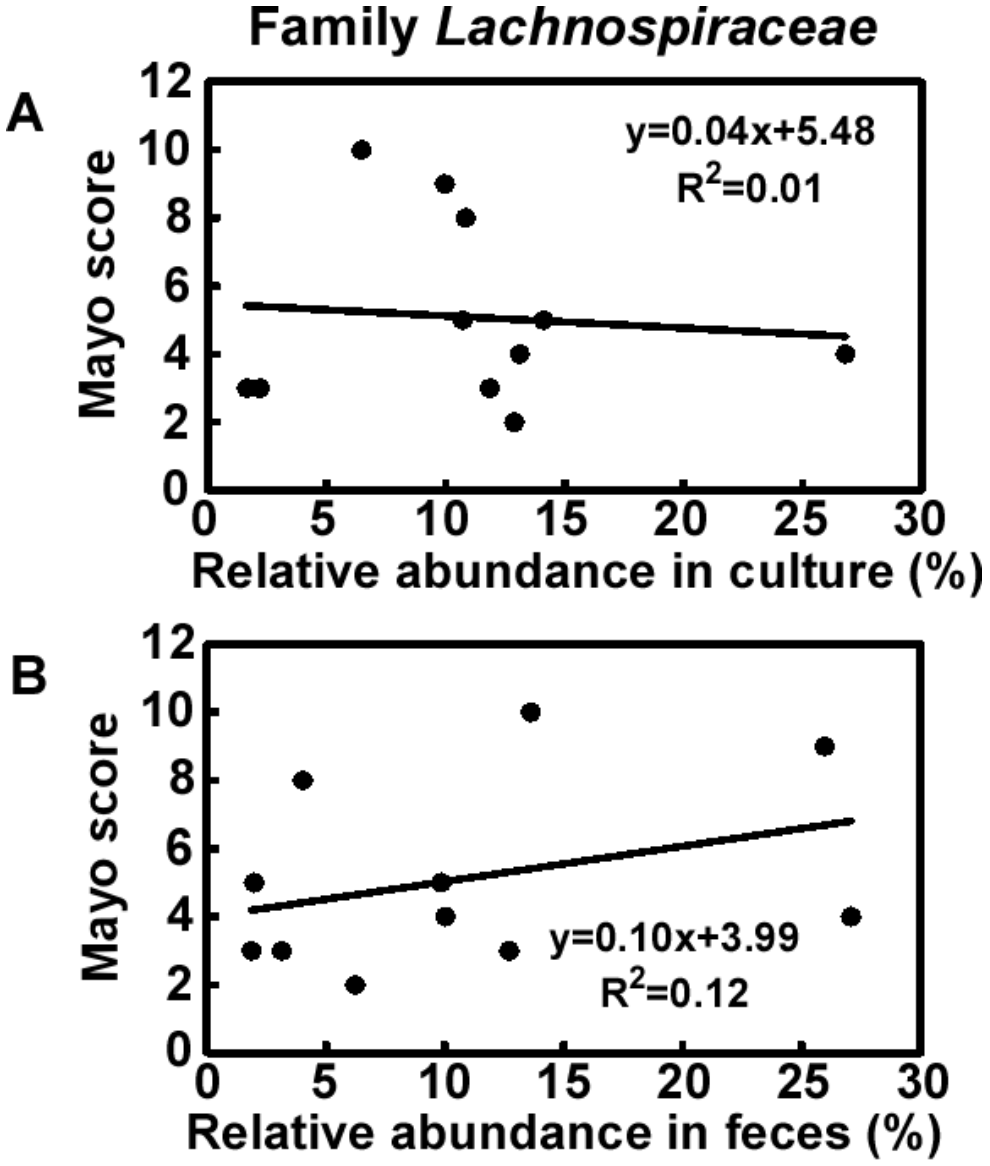


Figure S2.

Figure S2. Ratio of family *Lachnospiraceae* in fecal samples of healthy subjects (HS) and ulcerative colitis (UC) patients and corresponding fermentation cultures. Asterisks (*) indicate significant differences between HS and UC patients (**$P < 0.01$).



625 **Figure S3.**

626 **Figure S3.** Relation between family *Lachnospiraceae* and Mayo score. (A) Correlation
627 between UC Mayo score and relative abundance of family *Lachnospiraceae* in *in vitro* UC
628 cultures at 30 h after initiation of fermentation. (B) Correlation between UC Mayo score
629 and relative abundance of family *Lachnospiraceae* in UC fecal samples. The solid line and
630 its equation indicate the best-fit linear relationship.

631

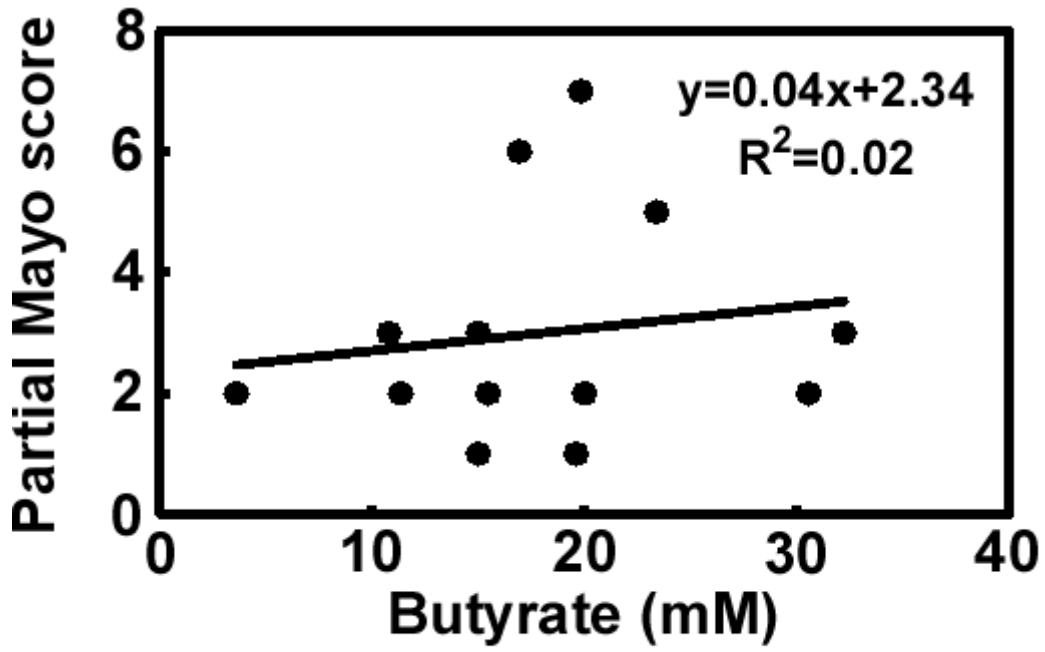
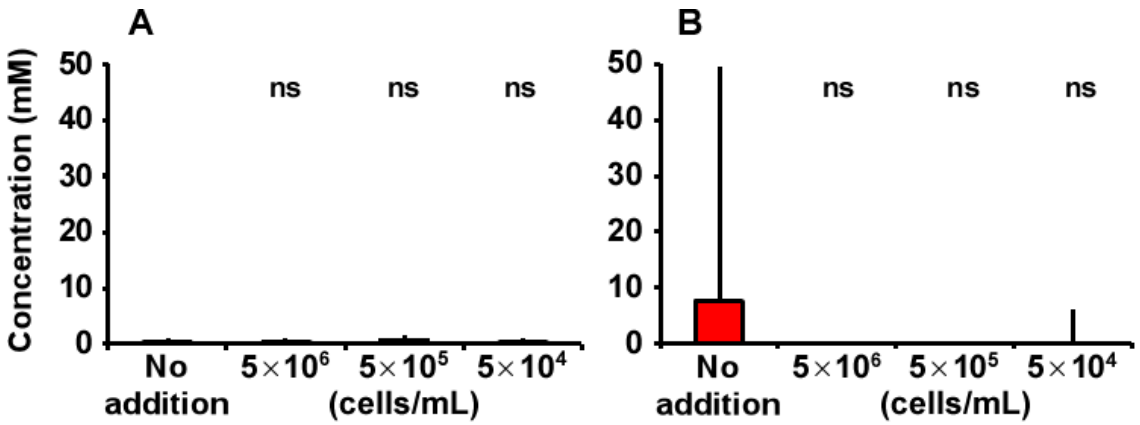


Figure S4.

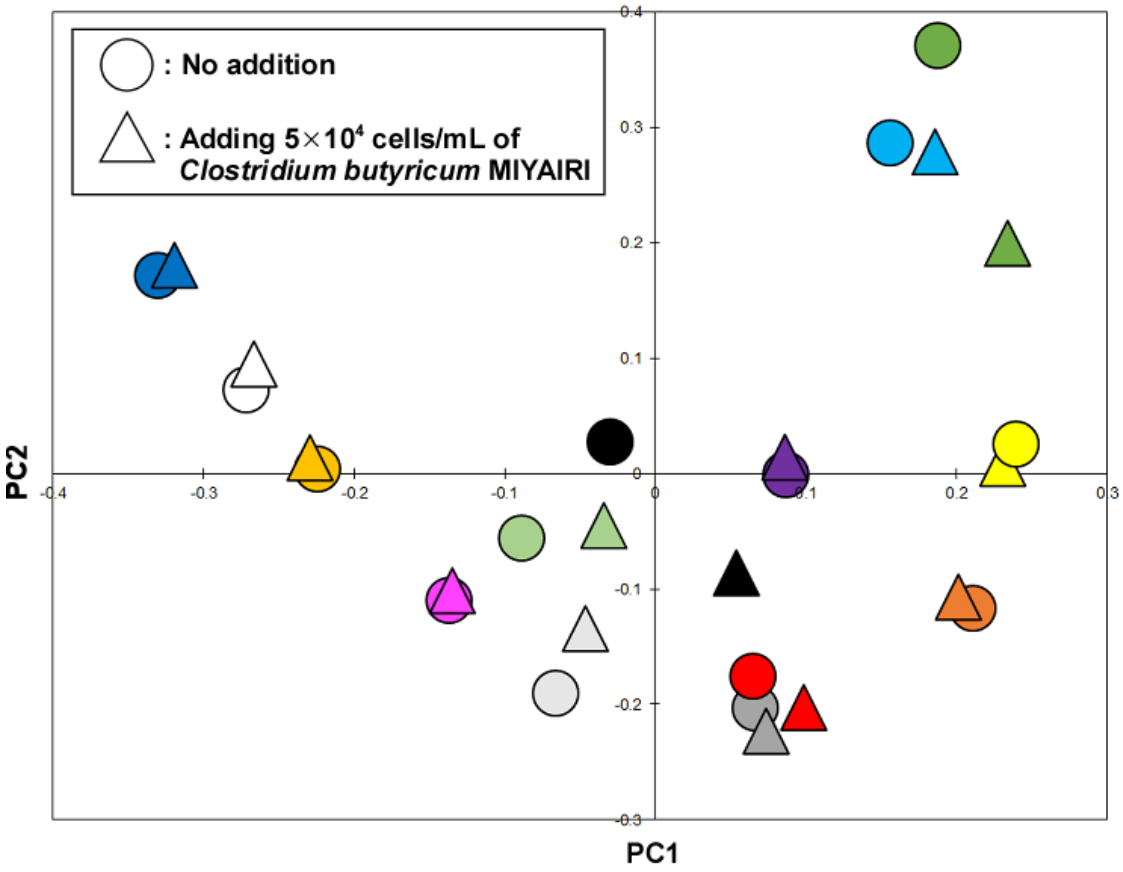
Figure S4. Correlation between butyrate production (mM) in *in vitro* ulcerative colitis (UC) cultures and partial Mayo score of UC patients. Solid lines and corresponding equations indicate the best-fit-linear relationship.



637 Figure S5.

638 **Figure S5.** Effect of addition of *Clostridium butyricum* MIYAIRI into the KUHIMM. The
639 cultures were sampled after 30 h of fermentation. Production of (A) succinate and (B)
640 lactate. ns indicates no significant differences between the addition of *C. butyricum*
641 MIYAIRI and no addition.

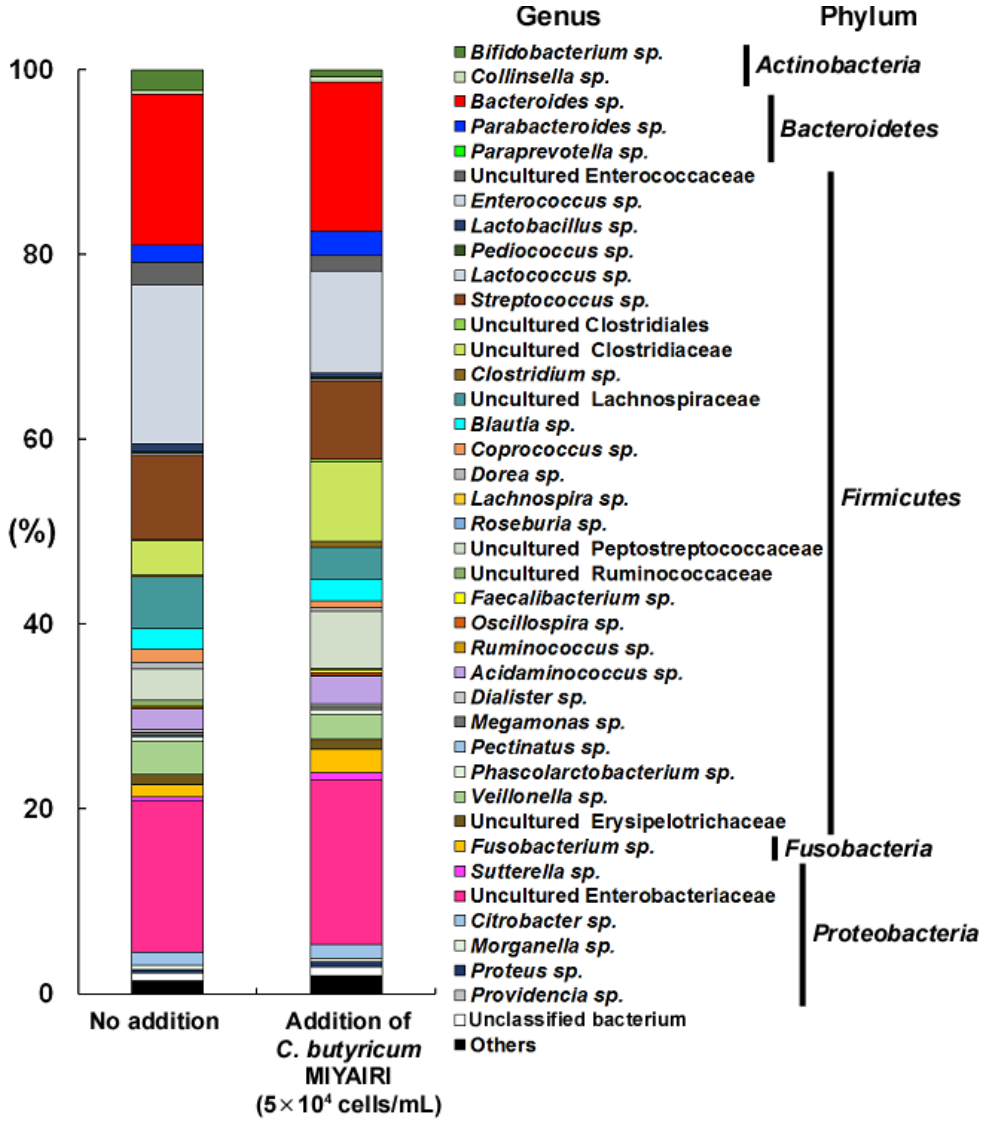
642



643 **Figure S6.**

644 **Figure S6.** Principal coordinate analysis (PCoA) of bacterial 16S rRNA gene data. Score
645 plot shows data using fermentation cultures without adding *Clostridium butyricum*
646 MIYAIRI (circle) and those adding 5 × 10^4 cells/mL of *C. butyricum* MIYAIRI (triangle) in
647 the KUHIMM system. Sampling was performed at 30 h after inoculating each fecal donor of
648 ulcerative colitis (UC) patients (n = 14) and initiating fermentation. PC1 and PC2 explain
649 12.8% and 9.88% of the variance, respectively. Differences in colors indicate differences in
650 UC patients.

651



652 **Figure S7.**

653 **Figure S7.** Genus-level compositional view of bacteria. 16S rRNA genes were evaluated in
654 cultures of ulcerative colitis (UC) patients (without adding *Clostridium butyricum* MIYAIRI)
655 and those adding 5 × 10^4 cells/mL of *C. butyricum* MIYAIRI in the KUHIMM system.
656 Sampling was conducted at 30 h after initiation of fermentation. Genera of lower
657 abundance were included in "Others".