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Cultured fecal microbial community and its impact as fecal microbiota transplantation treatment in mice gut inflammation

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Abstract

The fecal microbiome is identical to the gut microbial communities and provides an easy access to the gut microbiome. Therefore, fecal microbial transplantation (FMT) strategies have been used to alter dysbiotic gut microbiomes with healthy fecal microbiota, successfully alleviating various metabolic disorders, such as obesity, type 2 diabetes, and inflammatory bowel disease (IBD). However, the success of FMT treatment is donor-dependent and variations in gut microbes cannot be avoided. This problem may be overcome by using a cultured fecal microbiome. In this study, a human fecal microbiome was cultured using five different media; growth in brain heart infusion (BHI) media resulted in the highest microbial community cell count. The microbiome (16S rRNA) data demonstrated that the cultured microbial communities were similar to that of the original fecal sample. Therefore, the BHI-cultured fecal microbiome was selected for cultured FMT (cFMT). Furthermore, a dextran sodium sulfate (DSS)-induced mice-IBD model was used to confirm the impact of cFMT. Results showed that cFMT effectively alleviated IBD-associated symptoms, including improved gut permeability, restoration of the inflamed gut epithelium, decreased expression of pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1, IL-6, IL-12, and IL-17), and increased expression of anti-inflammatory cytokines (IL-4 and IL-10). Thus, study's findings suggest that cFMT can be a potential alternative to nFMT.

Key points

- *In vitro fecal microbial communities were grown in a batch culture using five different media.*
- *Fecal microbial transplantation was performed on DSS-treated mice using cultured and normal fecal microbes.*
- *Cultured fecal microbes effectively alleviated IBD-associated symptoms.*

Keywords Fecal microbiome · Cultured fecal microbes · Fecal microbial transplantation · Gut inflammation · Microbial therapeutics

Introduction

The human gut harbors an enormous number of microbes (3.8×10^{13}), primarily located in the colon known as gut microbes (Sender et al. 2016). Studies have established that the gut microbiota works as a virtual organ and participates in various metabolic activities, including the regulation of gut inflammation and maintenance of gut-barrier function. The human gut-microbial community is a composition of microbes belonging mainly to the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, and *Fusobacteria* (Hou et al. 2022). All together, these commensals, which include symbiotic, probiotic, and opportunistic pathogenic microbes, constitute the dynamic healthy gut microbiome. A dysbiotic gut shows a lack of

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microbial diversity leading to weakened or limited community interaction; this can increase the abundance of opportunistic pathogens and shift the microbiome-associated regulation of metabolism, resulting in metabolic disorders. Furthermore, past studies have shown that a diet lacking in microbiota-accessible carbohydrates (prebiotics) can lead to irreversible low microbial diversity in the gut and, thus, host vulnerability to various metabolic disorders (Sonnenburg et al. 2016). In addition, the use of antibiotics and exposure to pathogens, especially hospital-borne pathogens such as *Clostridium difficile*, *Clostridium botulinum*, and *Salmonella* spp., can also reduce the gut's microbial diversity (Bobo and Dubberke 2010). Particularly in the case of antibiotic-resistant *C. difficile* infection, which disrupts the gut microbiome and integrity of the colonic epithelial barrier, inducing life-threatening diarrhea. Similarly, patients with other metabolic diseases, such as inflammatory bowel disease (IBD) and diabetes, are often reported to have dysbiosis, wherein FMT can be used to alleviate such ailments, as it establishes the healthy gut microbial communities (Goloshchapov et al. 2019). A healthy gut microbiome lowers the lower gut inflammation, reduces the insulin resistance and obesity, and thus controls the metabolic disorders. Therefore, with time, FMT techniques are also used to treat certain metabolic disorders such as obesity (Yu et al. 2020) and gut inflammation (Zhang et al. 2020).

The fecal microbial communities are identical to the gut microbial communities and can be used to replenish the dysbiotic gut. These collected fecal microbial communities can be established in a dysbiotic gut as an oral gavage, and this technique is called fecal microbiota transplantation (FMT). Recently, FMT has been successfully used to treat the different metabolic and pathological disorders, such as IBD (Colman and Rubin 2014; Costello et al. 2019; Haifer et al. 2022), diabetes (de Groot et al. 2021; Ng et al. 2022), and *C. difficile*-induced diarrhea (Hamilton et al. 2013; Weingarden et al. 2015). In FMT, a patient's dysbiotic gut microbiome is replaced by a healthy donor's gut microbiome (Khoruts et al. 2021). Thus, an established healthy gut microbiome maintains the integrity of the epithelial barrier and regulates the inflammatory and immunological responses (Mishra et al. 2023). Essentially, the success of FMT is donor-dependent and sometimes causes concerns (He et al. 2022), as microbial diversity and the quality of the donated fecal microbiome cannot be controlled and standardized. Additionally, repeated FMTs increase the level and duration of microbial transplantation (Shimizu et al. 2016), but microbial variation in donor-dependent FMT can decrease the efficacy of FMT treatment. Such donor-dependent limitations of regular FMT can be addressed using cultured fecal-microbial communities, as microbial communities of a cultured batch offer a similar microbial diversity. Other than that, the cultured fecal microbial community also offers microbial communities without any variation in microbial diversity, making them even suitable for repetitive use for a patient.

In present study, the human fecal microbes were cultured in vitro using batch culture with five different media: brain heart infusion (BHI), fastidious anaerobe broth (FAB), reinforced clostridial broth (RCM), Schaedler anaerobe broth (SAM), and tryptic soy broth (TSB). Subsequently, the respective microbial communities were analyzed using next-generation sequencing. The results of cultured fecal FMT (cFMT) were compared with those of normal FMT. In addition, cFMT's efficiency in alleviating dextran sulfate sodium (DSS)-induced gut inflammation in a murine model was also investigated.

Materials and methods

Ethical permission and selection for fecal microbiota transplantation donor.

Ethical approval for the collection of the human-derived materials (stool) was obtained from the Institutional Review Board of Kyungpook National University (KNU) (KNU-2019–0129). A healthy donor was selected based on a questionnaire prepared by the Division of Gastroenterology, Department of Internal Medicine, Kyungpook National University Hospital, Daegu, South Korea. In addition, the animal procedures were approved by the Institutional Animal Care and Use Committee of the Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF-19100801–00).

Fecal sample collection and anaerobic cultivation

The FMT donor was selected on the basis of the standard operation procedure and pre-designed criteria (Kelly et al. 2017). Once a healthy donor was selected, a fecal sample was collected using a FECAL TRANSWAB (MW168S; Medical Wire & Equipment Co., Ltd., Corsham, UK) as per the given instructions. The collected stool sample was immediately transferred to an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI, USA), pre-maintained at aseptic and anaerobic conditions (> 20 ppm of oxygen). The fecal material was diluted tenfold (w/v) with sterile 20% glycerol and 0.85% saline in the anaerobic chamber and then filtered to eliminate any particulate substances. Five microbial media widely used in anaerobic cultivation were selected to establish single-batch anaerobic cultivation of the stool samples. The media used were BHI and TSB (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), FAB and RCM (KisanBio Co., Ltd., Seoul, South Korea), and SAM (Neogen Corp., Lansing, MI, USA). An oxygen scavenger, sodium sulfide (0.05%), was also added to each medium. Furthermore, the culture media were aseptically

sparged with nitrogen (N_2) gas and sealed to maintain the anaerobic conditions. The air-tight sealed culture media were then sterilized at 121 ℃ for 15 min, followed by cooling at room temperature until use (Speers et al. 2009).

In vitro anaerobic fecal microbiota cultivation was conducted in 45 mL of each of the sterilized media (BHI, FAB, RCM, SAM, and TSB) in triplicate. The collected stool aliquot was thawed on ice and 450 µL was inoculated into each media to a final concentration of 1% (v/v). After that, cultivation was anaerobically performed for 72 h at 36.5 ℃ and 180 rpm; samples were collected every 3 h from inoculation to 24 h to obtain the growth curve. Subsequently, sample collection was done once a day at 48 and 72 h from inoculation. For the downstream procedures, the collected samples were stored at − 80 ℃ until use. The inoculation and sample collection procedures were performed by puncturing the rubber stopper using a sterile disposable syringe while maintaining aseptic conditions.

Animal experiments

Seven-week-old C57BL/6 J male mice (average weight 20 g) were obtained from DooYeol Biotech, Seoul, South Korea, and maintained under specified stress- and pathogen-free conditions at 22 ℃, 50–60% humidity, and a 12-h light and dark cycle. The five experimental groups were as follows: control (untreated), dextran sodium sulfate (DSS)-treated only, and DSS treatment with normal mouse FMT (nFMT), DSS with original human FMT (hFMT), and DSS with cultured human FMT (cFMT). Mice groups, in triplicate, were placed in separate cages and had access to food and water ad libitum. To establish the mouse-IBD model, 2% (w/v) DSS (MP Biomedicals LCC, Irvine, CA, USA) was administered for 7 days; the mice were allowed to recover for the subsequent 2 days. The animal's health conditions were continuously monitored throughout the process.

Mucus and FMT administration in mice model

Mucus transplantation Mucus transplantation was conducted the day after the termination of the DSS treatment (day 8) using a previously described method (Burrello et al. 2018). The mucus used for transplantation was obtained by scraping the colon of sacrificed normal mice. The collected mucus was diluted with phosphate saline buffer (PBS) at a 1:1 ratio and was administered orally to the DSS only and DSS with nFMT, hFMT, or cFMT groups.

The DSS treatment induces the chemogenic lysis of gut epithelium and loss of mucus layer, thus causing the gut-inflammation. FMT treatment can establish the microbial community but thinner mucus layer also enhances the inflammation. Therefore, to nullify the role of mucus layer, oral mucus layer was given as per the established method (Burrello et al. 2018).

Fecal microbiota transplantation The fecal sample for the nFMT was obtained from the normal mice group; after dilution with 0.85% saline, the sample contained approximately 10^9 microbial cells/mL. The hFMT and cFMT were prepared using the same method as for the mouse stool. In the DSS group, a simulated treatment of 0.85% saline was administered to minimize any background impact. The mice in each of the FMT groups were given 200 µL of oral gavage daily for three consecutive days (days 9 to 11).

Colon and blood sample collection

After completing the DSS administration, colon and spleen samples were collected from the untreated and DSS-treated mice groups to confirm the inflammatory reaction of IBD. Anesthetized mice were sacrificed by cervical dislocation and a ventral midline incision was performed. Blood samples were collected to test the epithelial permeability and cytokine levels. The colon length and the weight of the spleen were recorded.

Intestinal epithelial permeability and hematoxylin and eosin assay

To determine the epithelial integrity of the colon, the fluorescein isothiocyanate-conjugated dextran (FITC-dextran) method was used, as colonic permeability is represented by the FITC-dextran concentration (Voetmann et al. 2023). Therefore, the day before sacrifice, FITC-dextran dissolved in PBS (100 mg/mL) was orally administered to mice (440 mg/kg of body weight). The samples' FITC-dextran level was evaluated using a spectrophotometer with an excitation wavelength of 485 nm and an emission wavelength of 528 nm (Gupta and Nebreda 2014).

In addition, the colonic morphology was studied using hematoxylin and eosin (H&E) staining. Colon samples of around 0.5 cm in length were opened longitudinally and the mucosa was rolled outward. After incubation in 10% formalin for 16 h at 4 °C, the tissues were embedded on paraffin plates, and H&E staining was carried out following the standard method (Cardiff et al. 2014).

Impact on cytokine levels

The cytokine levels in blood serum were determined using reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR). First, ribonucleic acid (RNA) was extracted from blood serum using the TRIzol method (Rio et al. 2010) and then purified and synthesized into complementary deoxyribonucleic acid (cDNA) (Urushima et al. 2015). The

mice mRNA levels of tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), and interleukin-17 (IL-17) were analyzed using delta-CT method and compared with the levels of eukaryotic translation elongation factor-2 (EEF2) as a reference. The primer information is provided in the supplementary table (Supplemental Table S1).

Next-generation sequencing

Genomic DNA was extracted from the human, mice, and cultured fecal samples using the QIAamp Powerfecal Pro DNA Kit (Qiagen, GmbH, Hilden, Germany), following the manufacturer's instructions. For the amplicon sequencing, the V4–V5 region of the 16S rRNA genes was amplified using 515F (5′-GTG NCA GCM GCC GCG GTR A-3′) and 907R (5′-CCG YCW ATT YHT TTR AGT TT-3′) as forward and reverse primers, respectively. The Illumina MiSeq library was prepared using an Illumina Nextera XT Library Preparation Kit (Illumina Inc., San Diego, CA, USA), following the manufacturer's instructions. The amplicon library's size, quality, and quantity were confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA) before running the sequencing platform. Amplicon sequencing was performed with 2 × 301 paired-end configurations; base calling and analysis were performed using MiSeq Control Software (Illumina Inc., San Diego, CA, USA). Sequencing was done at KNU NGS center (Daegu, South Korea).

Microbiome data analysis

The 16S rRNA amplicon sequencing data were analyzed using QIIME 2 (version 2023.9; https://forum.qiime2.org/) software. Furthermore, data processing to demultiplex and denoise the reads was achieved using the DADA2 plugin (https://github.com/benjjneb/dada2) in QIIME2, and chimeric sequences were removed (Callahan et al. 2016). For quality trimming, 20 bases were trimmed from the beginning of each sequence, and sequences longer than 260 lengths were truncated to remove the lower-quality sequences. Then, the identification of each amplicon sequence variant's (ASV) taxonomic details was performed using a classifier based on the SILVA release 138.1 database (https://www.arb-silva.de/download/arb-files/) (Quast et al. 2012). Further microbial diversity indices were analyzed using R computing language packages (https://www.R-project.org).

Statistics and data visualization

The microbial diversity was calculated using the vegan R package (Dixon 2003). Alpha diversity was calculated using the Chao and Shannon indices for the microbial richness and microbial evenness of the microbial diversity, respectively. Beta diversity was visualized using principal coordinate analysis (PCoA) to show the distribution of microbial communities and the variation between sample groups. For PCoA analysis, unweighted UniFrac distance metric (https://pypi.org/project/unifrac/) was used. In addition, microbial taxonomic analysis, including the phylum, family, and genus, was conducted using the Wilcoxon rank-sum and Kruskal–Wallis tests. These non-parametric tests were employed to determine whether the abundance of ASV differed among treatments in the taxonomy analysis. Analysis of variance (ANOVA) was to identify any significant differences ($p < 0.05$) between the groups.

Results

Cultured human fecal microbiome composition

The number of bacterial cells at inoculation (0 h) of each anaerobic culture media was around 10^7 cells/mL; this increased to ~ 10^9 cells/mL within 24 h of inoculation and remained maintained up to 72 h (Fig. 1a). Among the cultured media at 72 h after inoculation, the BHI, FAB, and TSB showed a significantly higher ($p < 0.0001$) count of bacterial cells compared with the RCM and SAM. Overall, 72-h culturing in BHI resulted in the highest microbial growth of all the media cultured (Fig. 1b).

Microbiome analysis showed that microbial richness and diversity were highest in the BHI compared with the other cultured media after 72 h of incubation. However, all cultured stool samples had lower diversity than the original stool (0 h) (Fig. 2a and b). The β-diversity results showed that the microbial communities of the BHI and FAB media at 72 h were most similar to that of the original stool sample (0 h) (Fig. 2c). In addition, the analysis showed a higher relative abundance of the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteriota* in all the cultured stool sample types (Fig. 3a). Further analysis showed that microbes of the families *Lachnospiraceae*, *Bifidobacteriaceae*, *Bacteroidaceae*, and *Selenomonadaceae* were most abundant in the original stool sample (0 h). In addition to these families, the cultured stool samples (48 and 72 h) had members of the families *Streptococcaceae*, *Clostridiaceae*, and *Lactobacillaceae* (Fig. 3b). At genus level, the original stool sample contained abundant microbes of the genera *Blautia*, *Bifidobacterium*, *Bacteroides*, *Fusicatenibacter*, *Megamonas*, and *Parabacteroides* (Fig. 3c), while the cultured stool groups' samples had abundant genera *Streptococcus* and *Paraclostridium* but lower abundance of the genus *Blautia*, which was exclusively abundant in original stool (Fig. 3c).

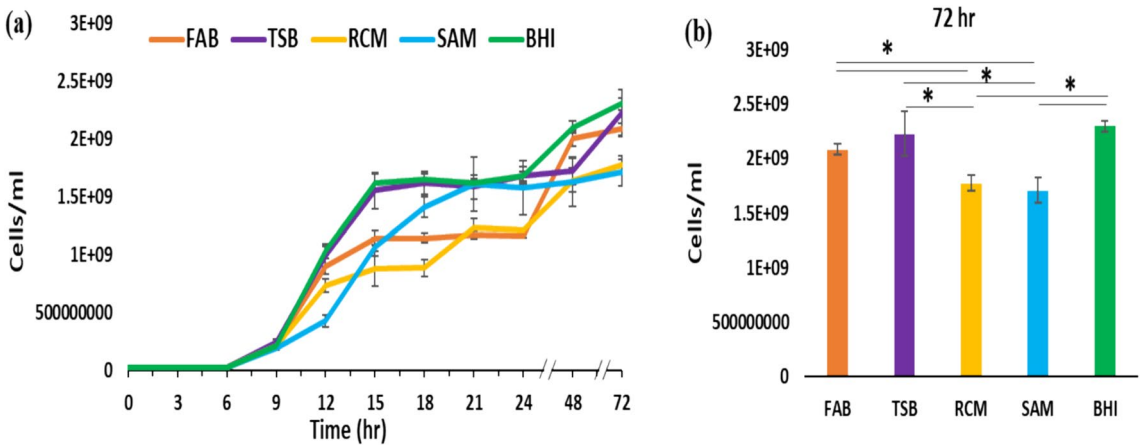

Fig. 1 Microbial growth curve of fecal microbial communities up to 72 h, in triplicate ($n=3$). (*BHI* brain heart infusion, *FAB* fastidious anaerobe broth, *RCM* reinforced clostridial broth, *SAM* Schaedler anaerobe broth, *TSB* tryptic soy broth)

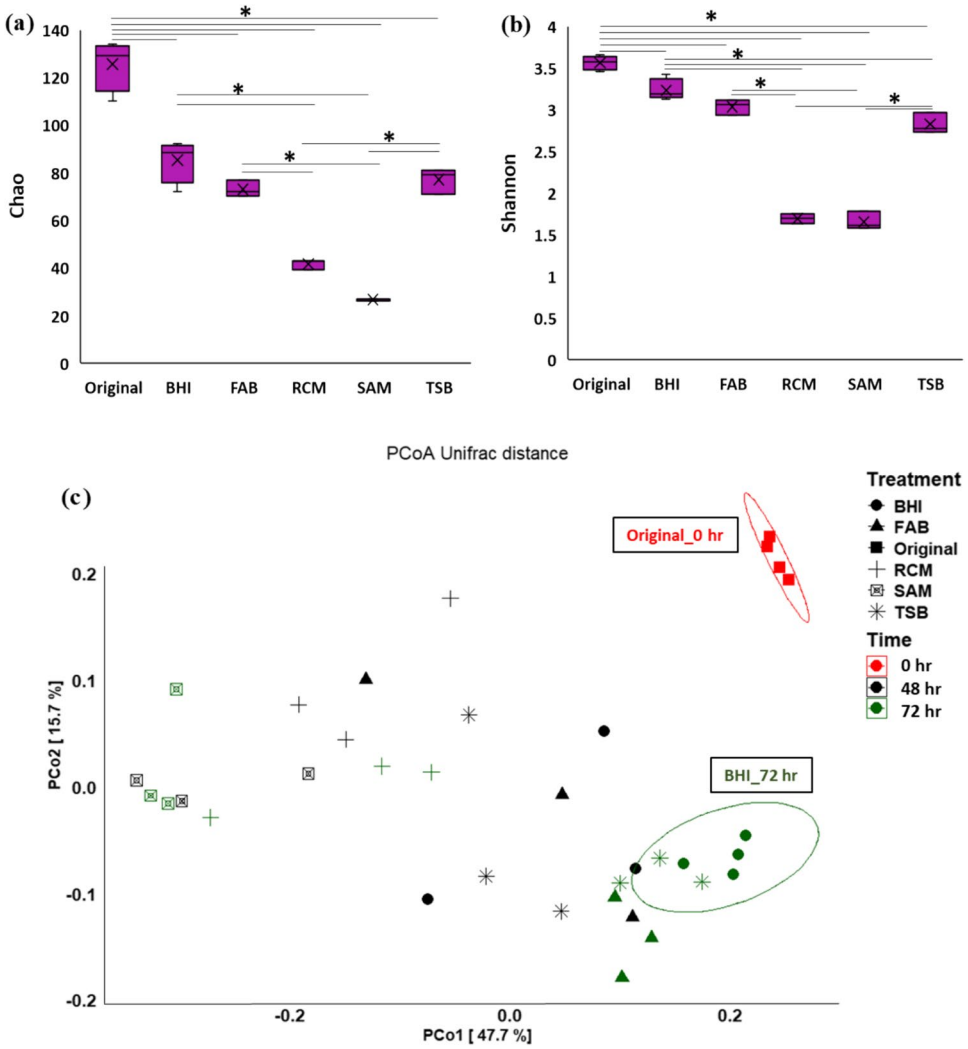

Fig. 2 Microbial diversity indices (α and β) of the fecal microbial communities (72 h): **a** microbial richness represented by the Chao index; **b** microbial diversity represented by the Shannon index; and **c** principal coordinate analysis (PCoA) plot for β-diversity showing community similarities between the sample cultured in brain heart infusion (BHI) medium (72 h) and the original stool sample (0 h). (*FAB* fastidious anaerobe broth, *RCM* reinforced clostridial broth, *SAM* Schaedler anaerobe broth, *TSB* tryptic soy broth)

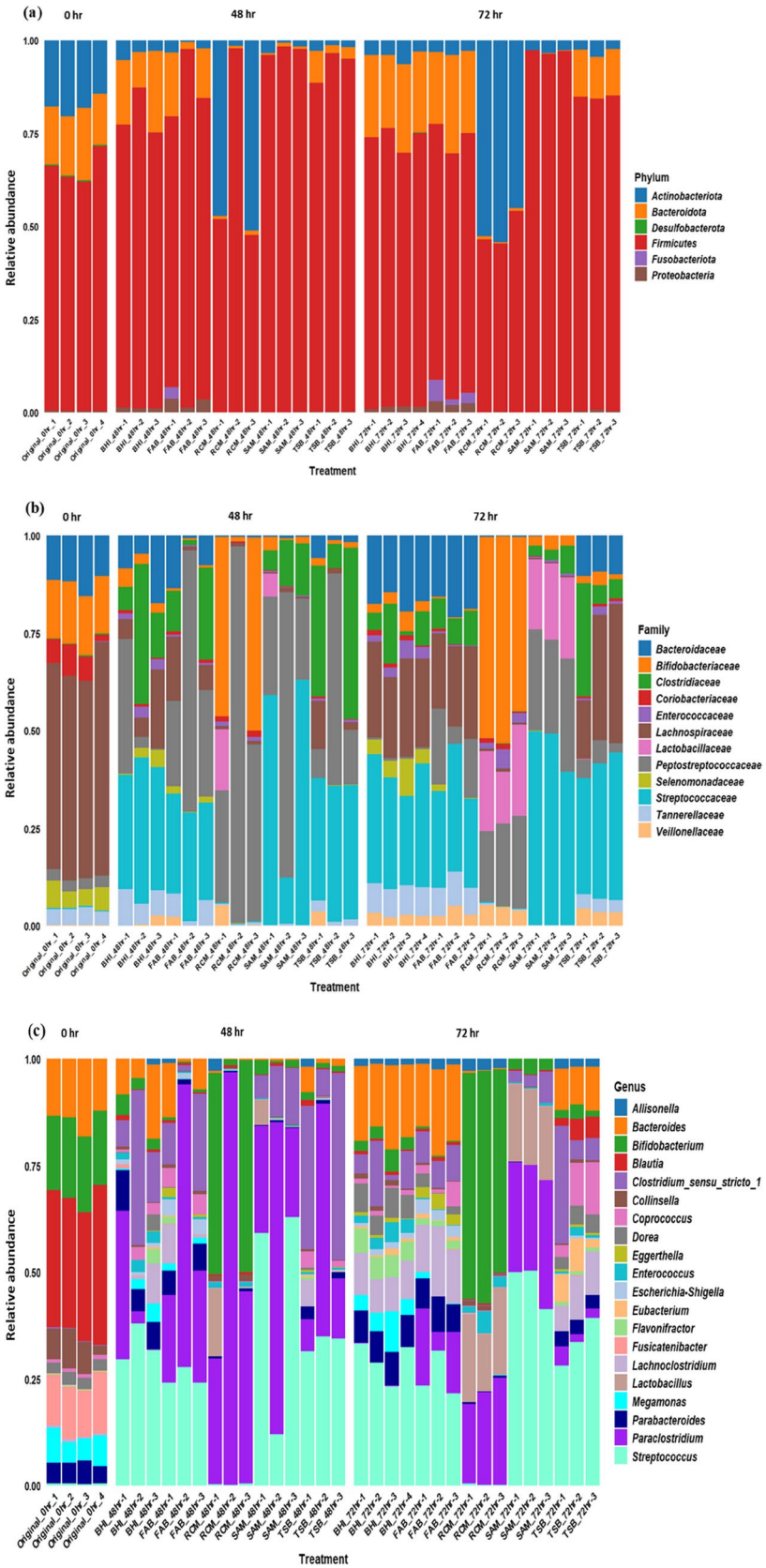

Fig. 3 Microbial taxonomy in cultured media at **a** phylum level, **b** family level, and **c** genus level. (*BHI* brain heart infusion, *FAB* fastidious anaerobe broth, *RCM* reinforced clostridial broth, *SAM* Schaedler anaerobe broth, *TSB* tryptic soy broth)

Dextran sodium sulfate-induced inflammation and the impact of fecal microbiota transplantation treatments

Seven consecutive days of DSS treatment caused a significant decrease in the mice's colon length and increased the spleen weight and colonic permeability, compared with the untreated group (Fig. S1 a–e). Likewise, H&E staining showed intact epithelial cells in the untreated group, while the DSS-treated group showed translocation of the immune cells into the colon and erosion of the epithelial cells (Fig. S1 f).

Impact of cultured human gut microbiota against DSS-induced inflammation

The samples collected after completion of FMT (15th day) showed a slight recovery in gut length compared with the untreated DSS group; however, the differences were not statistically significant (Fig. 4a and 4b). In addition, the gut permeability significantly decreased after FMT compared with the untreated DSS group (Fig. 4c). Other than that, spleen weights showed a significant increase in all the DSS treated groups, except nFMT and due to the loss of one spleen sample of cFMT group, ANOVA test cannot be performed for cFMT group to show the significant difference (Fig. 4e).

After 15 days of treatment, the H&E staining results showed the positive effects of FMT. The nFMT, cFMT, and hFMT groups showed recovery from colon inflammation, and their immune cells showed a relatively low level of immune cell translocation (Fig. 5). In addition, expression of genes for almost all the pro-inflammatory cytokines, including TNF-α, IFN, IL-12, and IL-17, was higher in the DSS-treated compared with the control groups (Fig. 6). The groups with DSS-induced inflammation that underwent FMT treatment were found to have a lower expression of inflammatory markers compared with the untreated DSS group, though IL-4 expression levels tended to be higher in the nFMT, cFMT, and hFMT-treated groups (Fig. 6).

Discussion

Healthy fecal microbiota has significant therapeutic potential as demonstrated by the successful use of FMT for recurrent *C. difficile* infections. Therefore, it was hypothesized that a balanced fecal microbial community could be used to alleviate various metabolic disorders, such as obesity, type 2 diabetes, and gut inflammation associated with the gut microbiota using FMT. However, FMT is donor-dependent and even two separately collected fecal samples from the same donor can have different microbiomes, which can affect the efficiency of the

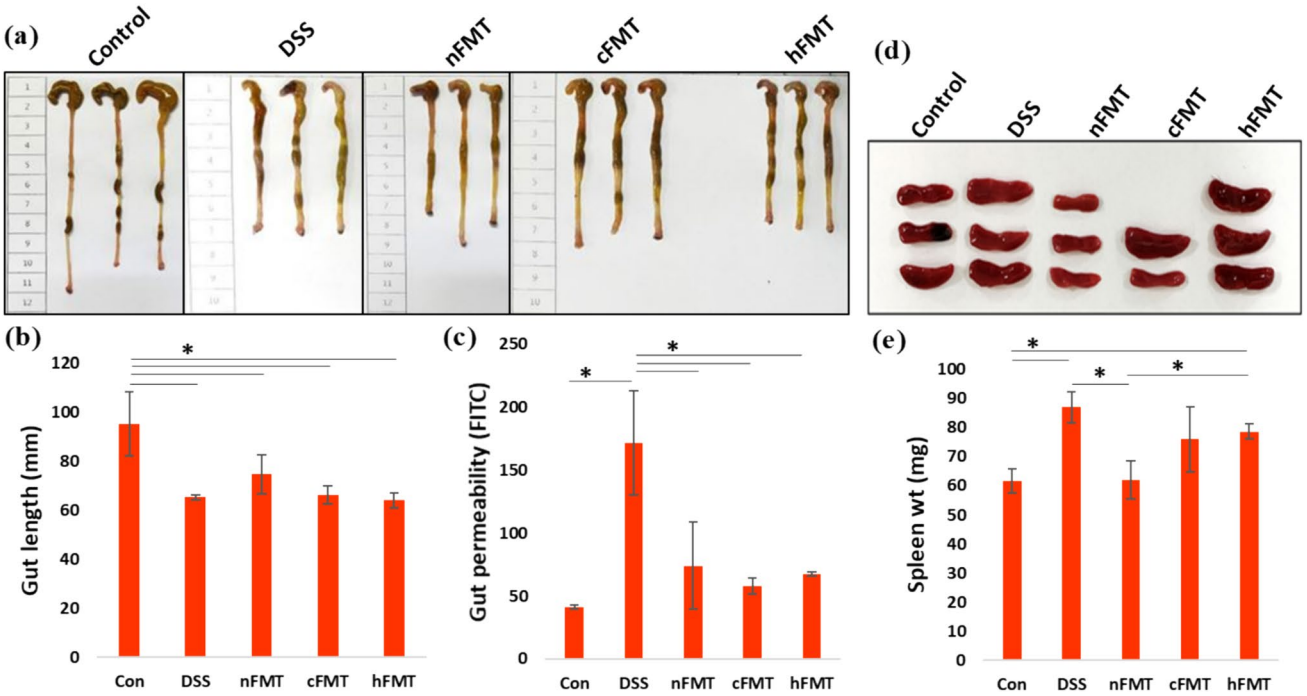

Fig. 4 Morphological changes in the colon after DSS-induced inflammation and subsequent FMT treatment: **a–b** significant decrease in the gut length after DSS treatment in all the groups including FMT groups; **c** significant decrease in gut permeability in the FMT groups; **d–e** higher spleen wt. in all DSS-treated groups including FMT groups. (cFMT, cultured human FMT; hFMT, human FMT; nFMT, normal mouse FMT) (significance; $*p < 0.05$)

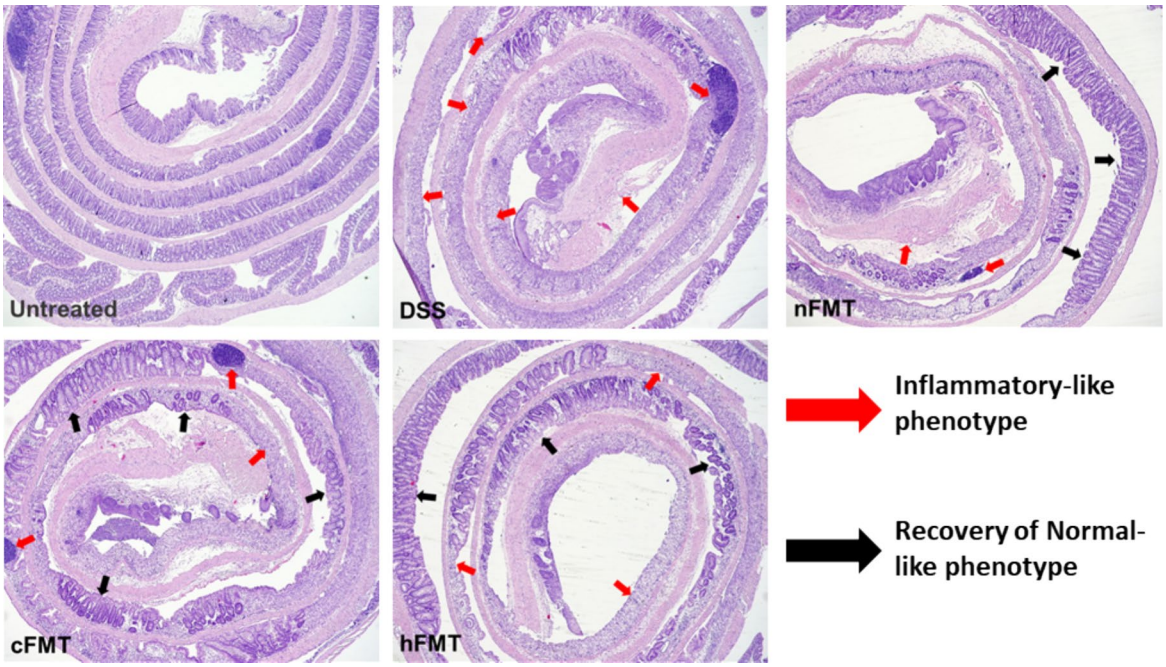

Fig. 5 Effect of FMT on the colon tissue of DSS-treated mice. Colonic tissues were collected after completing 3 days of FMT. The H&E staining results show the intact colonic lining of the following: control group, untreated or no DSS; DSS, DSS-induced inflammation without FMT; nFMT, DSS-induced inflammation + FMT with normal mouse feces; cFMT, DSS-induced inflammation + FMT with cultured human feces; hFMT, DSS-induced inflammation + FMT with human feces

treatment. Therefore, this study aimed to examine a cultured fecal microbiome and its suitability for use in FMT. Additionally, the selected media (BHI, FAB, RCM, SAM, and TSB) are the best-known complex nutrient media which can be used to grow the mix communities. Out of which, BHI is continuously used to grow the fecal microbes (Tao et al. 2023; Yousi et al. 2019). Therefore, in the present study, we focused on the BHI media and also compared the suitability of the other medias.

First, a fecal microbial community was collected from the stool of a healthy donor and cultured anaerobically using five different media (BHI, FAB, RCM, SAM, and TSB) for 72 h, where cultured BHI, TSB, and FAB media showed a significantly higher number of microbial cells (~ 10^9 cells/mL) compared with the RCM and SAM media.. Among the differently cultured stool samples, the BHI-grown microbial community (72 h) was the closest to that of the original stool (0 h) fecal microbiome compared with the other culture media's communities (FAB, RCM, SAM, and TSB). Similarly, β-diversity between the original stool and BHI-grown microbial communities (after 72 h) was closer compared with the samples cultured in the other media; these results demonstrated the microbial similarity between the BHI-grown and original fecal microbiomes. The samples cultivated for 72 h in the BHI and FAB media had microbiome similarity with the original stool sample at the phylum level, including abundant microbes of the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteriota*. However, the FAB-cultivated stool microbial community (72 h) was found to be abundant in bacteria from the phylum *Fusobacteriota*, which were absent in the original stool and BHI-cultivated fecal microbiomes. Therefore, the 72-h BHI-cultivated microbiome was the most similar to the original stool's (0 h) microbiome. Similarly, at the family level, only the original stool and BHI-cultivated fecal communities were abundant in members of the family *Selenomonadaceae*; microbes of the families *Lachnospiraceae*, *Bacteroidaceae*, and *Bifidobacteriaceae* were also commonly abundant. At genus level, only the 72-h BHI-cultivated microbiome was relatable at the genus level to the original stool (0 h); in addition, both showed an abundance of microbes of the genus *Parabacteroides*. These microbes are core to a healthy gut microbiome and confer a protective effect in the host's gut by producing short-chain fatty acids, which are found to be depleted in IBD patients (Cui et al. 2022). Additionally, only the 72-h BHI-cultivated stool microbial community had an abundance of genera *Bacteroides* and *Bifidobacterium*. These genera are important in a healthy gut microbiome; *Bacteroides* species benefit the host by metabolizing complex polysaccharides and glycans (Wexler and Goodman 2017) and *Bifidobacterium* play a probiotic and health-promoting role in the gut (O'Callaghan and Van Sinderen 2016). The 72-h FAB-cultivated fecal microbial community was abundant in bacteria from the genera *Bacteroides* but lacking in the genera *Bifidobacterium*. Overall, microbiome analysis showed that only BHI (72 h) had healthy, balanced, and gut microbial communities similar to the original stool

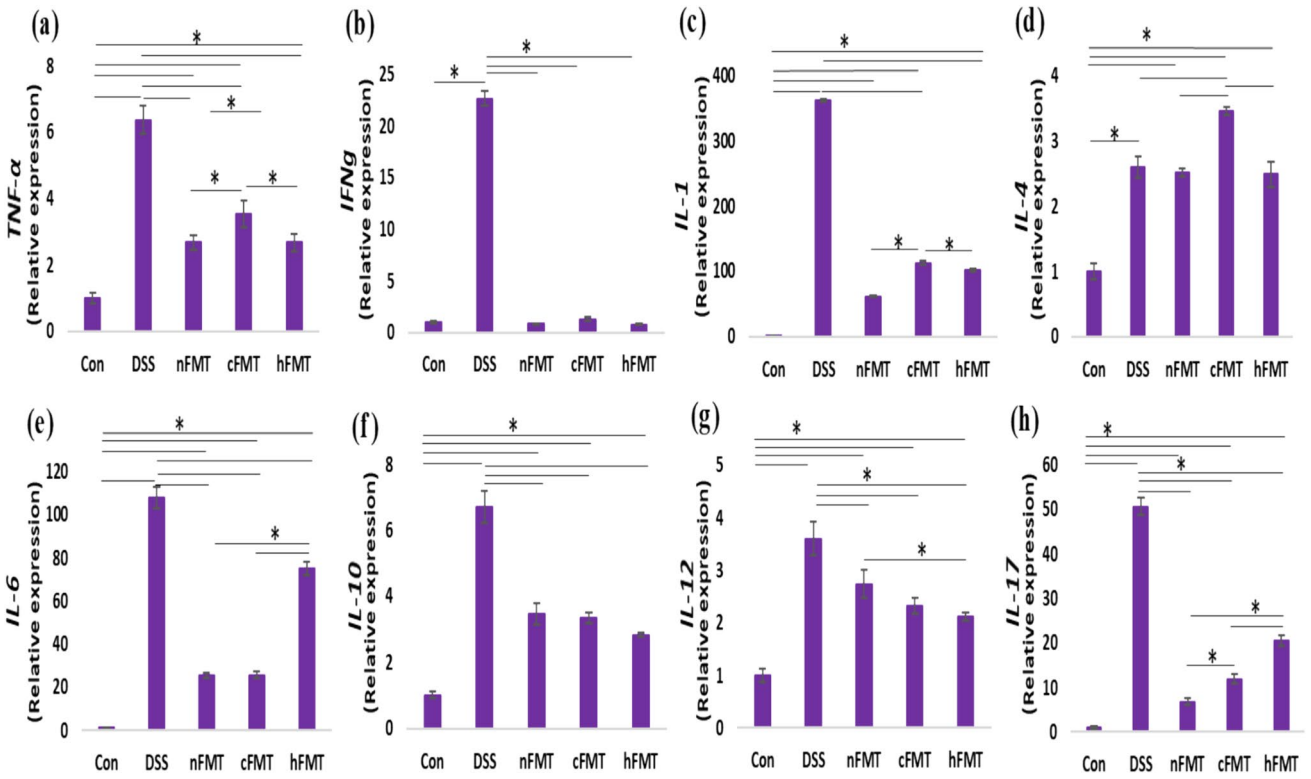

Fig. 6 Variations in pro-inflammatory (IFN-γ, TNF-α, IL-1, IL-6, IL-12, and IL-17) and anti-inflammatory markers (IL-4 and IL-10) in mice after 15 days. Con, control/untreated mice; DSS, DSS-induced inflammatory group; and DSS with FMT-treated groups. Mice received FMT (cFMT, cultured human FMT; hFMT, human FMT; nFMT, normal mouse FMT) between days 9 and 11 and subsequently 3 days to settle the FMT microbes in the mice's gut. Samples collected on day 15 showed higher levels of inflammatory markers in the DSS group compared with the control and FMT-treated groups (significance; * $p < 0.05$)

sample. Therefore, the 72-h BHI-cultured fecal community was identified as the most suitable for FMT compared with the microbiota cultivated in the other media (FAB, RCM, SAM, and TSB) and was selected for the mice FMT. This is in accordance with the various findings, which also support the suitability of the BHI media for the growth of fecal microbial communities (Shalon et al. 2023; Yousi et al. 2019).

Furthermore, DSS-induced IBD was established in a mouse model (Talley et al. 2021) to examine the therapeutic possibilities of FMT using a 72-h BHI-cultured fecal microbiome. The results confirmed that the DSS treatment significantly ($p < 0.05$) reduced the gut length and increased the gut permeability and spleen weight compared with the DSS-untreated mice (control group). These indicators are characteristic of mice colitis; shorter gut length and higher spleen weight result from the colonic cell damage caused by DSS-induced inflammation (Chassaing et al. 2014). In the DSS group, gut permeability was also compromised because DSS causes an alteration in occludin, a gut barrier protein found in colonic epithelial cells (Chelakkot et al. 2017). A compromised gut barrier function leads to a leaky gut situation, resulting from a higher lipopolysaccharide load passing into systemic circulation, which can stimulate systemic inflammation; leaky gut syndrome is frequently associated with IBD (Camilleri 2019). Additionally, the H&E staining results showed intact epithelial cells in the DSS-untreated group (Con), while the DSS-treated group showed translocation of the immune cells into the colon and erosion of the epithelial cells. These results are supported by previous findings confirming IBD-like symptoms established in the mouse gut (Talley et al. 2021).

After the FMT (day 15), the results showed no significant difference in the gut length of FMT-treated groups (cFMT, nFMT, and hFMT) compared with the DSS group; however, FMT was notably effective in limiting gut permeability, which is in harmony with the previous finding (Craven et al. 2020a). The cFMT, nFMT, and hFMT groups showed that the treatment had similar effects to each other, although nFMT was significantly effective in lowering the spleen weight. This might be because only the nFMT group had the mice-derived microbes but cFMT and hFMT had the human-derived microbes. Previous studies have confirmed that the gut microbiome is directly associated with gut permeability, and a healthy gut microbiome alleviates gut permeability (Mishra et al. 2023). In the case of gut dysbiosis or inflammation, FMT can improve the gut-barrier function by limiting gut permeability (Craven et al. 2020b; Li et al. 2024). Additionally, the

results of the H&E staining showed a greater deformation of the gut's cryptic structure and mucosal epithelium, and infiltration in mucosal layers compared with that of the control (DSS-untreated) group. The FMT group samples showed some mucosal structural damage but improved cryptic structures. In addition, the mucosal epithelium also showed lower inflammatory cell infiltrate, indicating signs of inflammation recovery. The histological examinations highlight that FMT from healthy donors and cultured feces had the similar ability to reduce inflammation and promote healing in DSS-induced colitis. This finding was supported by previous studies that demonstrated that DSS-induced disorientation of the gut tissues could be alleviated through FMT (Burrello et al. 2018). Furthermore, the RT-PCR results demonstrated a higher level of pro-inflammatory markers, including IFN-γ, TNF-α, IL-1, IL-6, IL-12, and IL-17, in the DSS group compared with the untreated control. The DSS-treated groups that underwent either nFMT, cFMT, or hFMT treatment were found to have lower DSS-induced inflammation. However, the expression of the pro-inflammatory markers was significantly higher ($p > 0.05$) in all the FMT-treated groups compared with the control group that was not administered DSS. This was because the FMT groups were subjected to DSS treatment before FMT and the induced inflammation was not completely reversed by FMT or the period of recovery was insufficient. A previous study confirmed that IFN-γ, TNF-α, IL-1, IL-6, IL-12, and IL-17 levels are linked with IBD, and higher levels are associated with the IBD progression (Friedrich et al. 2019; Ligumsky et al. 1990; Strober and Fuss 2011); moreover, DSS treatment has been reported to increase their levels (Kwon et al. 2021). Similarly, the anti-inflammatory cytokines (IL-10 and IL-4) levels were found to be higher in the DSS followed by FMT groups. Past studies have shown that FMT increased IL-10 levels, which can ultimately lead to reduced colonic inflammation (Burrello et al. 2018; Svačina et al. 2024). However, the DSS group that was not treated by FMT also showed a higher level of IL-10, which may indicate some insufficient recovery from the induced gut inflammation. In addition, the presence of anti-inflammatory IL-4 was higher in the FMT-treated groups (nFMT, cFMT, and hFMT) compared with the DSS group, suggesting that higher IL-4 levels are a result of FMT. In study, we observed that hFMT group is less effective than other FMT groups, which might be due to the fact that human microbiome is different form mice microbiome. On the other side, cFMT which is derived from the human fecal microbial community might be having certain microbial variation compared to original stool, making it microbially closer to the mice microbiome.

Overall, the study's findings suggest that cFMT was effective against IBD-associated symptoms, including improving gut permeability, restoring the inflamed gut epithelium, decreasing levels of pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1, IL-6, IL-12, and IL-17), and increasing levels of anti-inflammatory cytokines (IL-4 and IL-10). This suggests that cultured stool has enormous potential to be used in FMT as cFMT has potential therapeutic applications in treating gut inflammation and metabolic disease. The present work shows that fecal microbial communities can be grown synthetically and these cultured fecal communities can be used as microbial therapeutics in FMT. These cultured microbial communities or cFMT offer standardized-constant diversity of the fecal microbes making them suitable for the repetitive FMT, which has further significance in the treatment of various metabolic disorders including IBD. However, further research is needed to achieve results of pathologic significance for human use, as western blot or antibody assays can be done to confirm the protein expression.

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Author contribution VS analyzed the data, drafted the results, and wrote the manuscript; SDC, HS, HL, and YJL performed the experiments; VS and KM analyzed the microbiome data; ESK and JHS supervised, reviewed, and approved the manuscript.

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Data availability The Next-Generation Sequencing (NGS) data files have been submitted to the National Center for Biotechnology Information (NCBI) with BioProject ID PRJNA1121121.

Declarations

Ethics approval Ethical approval for the collection of the human-derived materials (stool) and animal experiment was obtained from the Institutional Review Board of Kyungpook National University (KNU) (KNU-2019–0129) and by the Institutional Animal Care and Use Committee of the Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF-19100801–00), respectively.

Competing interests The authors declare no competing interests.

References

Bobo LD, Dubberke ER (2010) Recognition and prevention of hospital-associated enteric infections in the intensive care unit. Crit Care Med 38(80):S324. https://doi.org/10.1097/CCM.0b013e3181e69f05

Burrello C, Garavaglia F, Cribiù FM, Ercoli G, Lopez G, Troisi J, Colucci A, Guglietta S, Carloni S, Guglielmetti S, Taverniti V, Nizzoli G, Bosari S, Caprioli F, Rescigno M, Facciotti F (2018) Therapeutic faecal microbiota transplantation controls intestinal inflammation through IL10 secretion by immune cells. Nat Commun 9(1):5184. https://doi.org/10.1038/s41467-018-07359-8

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from illumina amplicon data. Nat Methods 13(7):581–583. https://doi.org/10.1038/nmeth.3869

Camilleri M (2019) Leaky gut: mechanisms, measurement and clinical implications in humans. Gut 68(8):1516–1526. https://doi.org/10.1136/gutjnl-2019-318427

Cardiff RD, Miller CH (2014) Munn RJ (2014) Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harb Protoc 6:pdb. prot073411. https://doi.org/10.1101/pdb.prot073411

Chassaing B, Aitken JD, Malleshappa M, Vijay-kumar M (2014) Dextran sulfate sodium (DSS)-Induced colitis in mice. Curr Protoc Immunol 104(1):15.25.1-15.25.14. https://doi.org/10.1002/0471142735.im1525s104

Chelakkot C, Ghim J, Rajasekaran N, Choi J-S, Kim J-H, Jang MH, Shin YK, Suh P-G, Ryu SH (2017) Intestinal epithelial cell-specific deletion of pld2 alleviates dss-induced colitis by regulating occludin. Sci Rep 7(1):1573. https://doi.org/10.1038/s41598-017-01797-y

Colman RJ, Rubin DT (2014) Fecal microbiota transplantation as therapy for inflammatory bowel disease: a systematic review and meta-analysis. J Crohns Colitis 8(12):1569–1581. https://doi.org/10.1016/j.crohns.2014.08.006

Costello SP, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, Katsikeros R, Makanyanga J, Campaniello MA, Mavrangelos C (2019) Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. JAMA 321(2):156–164. https://doi.org/10.1001/jama.2018.20046

Craven L, Rahman A, Nair Parvathy S, Beaton M, Silverman J, Qumosani K, Hramiak I, Hegele R, Joy T, Meddings J, Urquhart B, Harvie R, McKenzie C, Summers K, Reid G, Burton JP, Silverman M (2020) Allogenic fecal microbiota transplantation in patients with nonalcoholic fatty liver disease improves abnormal small intestinal permeability: a randomized control trial. Am J Gastroenterol 115(7):1055–1065

Craven L, Rahman A, Parvathy SN, Beaton M, Silverman J, Qumosani K, Hramiak I, Hegele R, Joy T, Meddings J (2020b) Allogenic fecal microbiota transplantation in patients with nonalcoholic fatty liver disease improves abnormal small intestinal permeability: a randomized control trial. Am J of Gastroenterol 115(7):1055–1065. https://doi.org/10.14309/ajg.0000000000000661

Cui Y, Zhang L, Wang X, Yi Y, Shan Y, Liu B, Zhou Y, Lü X (2022) Roles of intestinal *Parabacteroides* in human health and diseases. FEMS Microbiol Lett 369(1). https://doi.org/10.1093/femsle/fnac072

de Groot P, Nikolic T, Pellegrini S, Sordi V, Imangaliyev S, Rampanelli E, Hanssen N, Attaye I, Bakker G, Duinkerken G, Joosten A, Prodan A, Levin E, Levels H, Potter van Loon B, van Bon A, Brouwer C, van Dam S, Simsek S, van Raalte D, Stam F, Gerdes V, Hoogma R, Diekman M, Gerding M, Rustemeijer C, de Bakker B, Hoekstra J, Zwinderman A, Bergman J, Holleman F, Piemonti L, De Vos W, Roep B, Nieuwdorp M (2021) Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. Gut 70(1):92. https://doi.org/10.1136/gutjnl-2020-322630

Dixon P (2003) VEGAN, a package of R functions for community ecology. J Veg Sci 14(6):927–930

Friedrich M, Pohin M, Powrie F (2019) Cytokine networks in the pathophysiology of inflammatory bowel disease. Immunity 50(4):992–1006. https://doi.org/10.1016/j.immuni.2019.03.017

Goloshchapov OV, Olekhnovich EI, Sidorenko SV, Moiseev IS, Kucher MA, Fedorov DE, Pavlenko AV, Manolov AI, Gostev VV, Veselovsky VA, Klimina KM, Kostryukova ES, Bakin EA, Shvetcov AN, Gumbatova ED, Klementeva RV, Shcherbakov AA, Gorchakova MV, Egozcue JJ, Pawlowsky-Glahn V, Suvorova MA, Chukhlovin AB, Govorun VM, Ilina EN, Afanasyev BV (2019) Long-term impact of fecal transplantation in healthy volunteers. BMC Microbiol 19(1):312. https://doi.org/10.1186/s12866-019-1689-y

Gupta J, Nebreda AR (2014) Analysis of intestinal permeability in mice. Bio Protoc 4(22):e1289–e1289

Haifer C, Paramsothy S, Kaakoush NO, Saikal A, Ghaly S, Yang T, Luu LDW, Borody TJ, Leong RW (2022) Lyophilised oral faecal microbiota transplantation for ulcerative colitis (LOTUS): a randomised, double-blind, placebo-controlled trial. Lancet Gastroenterol Hepatol 7(2):141–151. https://doi.org/10.1016/S2468-1253(21)00400-3

Hamilton MJ, Weingarden AR, Unno T, Khoruts A, Sadowsky MJ (2013) High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria. Gut Microbes 4(2):125–135. https://doi.org/10.4161/gmic.23571

He R, Li P, Wang J, Cui B, Zhang F, Zhao F (2022) The interplay of gut microbiota between donors and recipients determines the efficacy of fecal microbiota transplantation. Gut Microbes 14(1):2100197. https://doi.org/10.1080/19490976.2022.2100197

Hou K, Wu Z-X, Chen X-Y, Wang J-Q, Zhang D, Xiao C, Zhu D, Koya JB, Wei L, Li J, Chen Z-S (2022) Microbiota in health and diseases. Signal Transduct Target Ther 7(1):135. https://doi.org/10.1038/s41392-022-00974-4

Kelly CR, Kim AM, Laine L, Wu GD (2017) The AGA's fecal microbiota transplantation national registry: an important step toward understanding risks and benefits of microbiota therapeutics. Gastroenterology 152(4):681–684. https://doi.org/10.1053/j.gastro.2017.01.028

Khoruts A, Staley C, Sadowsky MJ (2021) Faecal microbiota transplantation for *Clostridioides difficile*: mechanisms and pharmacology. Nat Rev Gastroenterol Hepatol 18(1):67–80. https://doi.org/10.1038/s41575-020-0350-4

Kwon J, Lee C, Heo S, Kim B, Hyun C-K (2021) DSS-induced colitis is associated with adipose tissue dysfunction and disrupted hepatic lipid metabolism leading to hepatosteatosis and dyslipidemia in mice. Sci Rep 11(1):5283. https://doi.org/10.1038/s41598-021-84761-1

Li D, Liang W, Zhang W, Huang Z, Liang H, Liu Q (2024) Fecal microbiota transplantation repairs intestinal permeability and regulates the expression of 5-HT to influence alcohol-induced depression-like behaviors in C57BL/6J mice. Front Microbiol 14. https://doi.org/10.3389/fmicb.2023.1241309

Ligumsky M, Simon PL, Karmeli F, Rachmilewitz D (1990) Role of interleukin 1 in inflammatory bowel disease–enhanced production during active disease. Gut 31(6):686. https://doi.org/10.1136/gut.31.6.686

Mishra SP, Wang B, Jain S, Ding J, Rejeski J, Furdui CM, Kitzman DW, Taraphder S, Brechot C, Kumar A, Yadav H (2023) A mechanism by which gut microbiota elevates permeability and inflammation in obese/diabetic mice and human gut. Gut 72(10):1848. https://doi.org/10.1136/gutjnl-2022-327365

Ng SC, Xu Z, Mak JWY, Yang K, Liu Q, Zuo T, Tang W, Lau L, Lui RN, Wong SH, Tse YK, Li AYL, Cheung K, Ching JYL, Wong VWS, Kong APS, Ma RCW, Chow EYK, Wong SKH, Ho ICH, Chan PKS, Chan FKL (2022) Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: a 24-week, double-blind, randomised controlled trial. Gut 71(4):716. https://doi.org/10.1136/gutjnl-2020-323617

O'Callaghan A, Van Sinderen D (2016) *Bifidobacteria* and their role as members of the human gut microbiota. Front Microbiol 7:206360. https://doi.org/10.3389/fmicb.2016.00925

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41(D1):D590–D596. https://doi.org/10.1093/nar/gks1219

Rio DC, Ares M, Hannon GJ (2010) Nilsen TW (2010) Purification of RNA using TRIzol (TRI Reagent). Cold Spring Harb Protoc 6:pdb.prot5439. https://doi.org/10.1101/pdb.prot5439

Sender R, Fuchs S, Milo R (2016) Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 14(8):e1002533. https://doi.org/10.1371/journal.pbio.1002533

Shalon D, Culver RN, Grembi JA, Folz J, Treit PV, Shi H, Rosenberger FA, Dethlefsen L, Meng X, Yaffe E, Aranda-Díaz A, Geyer PE, Mueller-Reif JB, Spencer S, Patterson AD, Triadafilopoulos G, Holmes SP, Mann M, Fiehn O, Relman DA, Huang KC (2023) Profiling the human intestinal environment under physiological conditions. Nature 617(7961):581–591. https://doi.org/10.1038/s41586-023-05989-7

Shimizu H, Arai K, Abe J, Nakabayashi K, Yoshioka T, Hosoi K, Kuroda M (2016) Repeated fecal microbiota transplantation in a child with ulcerative colitis. Pediatr Int 58(8):781–785. https://doi.org/10.1111/ped.12967

Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL (2016) Diet-induced extinctions in the gut microbiota compound over generations. Nature 529(7585):212–215. https://doi.org/10.1038/nature16504

Speers AM, Cologgi DL, Reguera G (2009) Anaerobic cell culture. Curr Protoc Microbiol 12(1):A.4F.1-A.4F.16. https://doi.org/10.1002/9780471729259.mca04fs12

Strober W, Fuss IJ (2011) Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. Gastroenterology 140(6):1756-1767.e1. https://doi.org/10.1053/j.gastro.2011.02.016

Svačina MKR, Gao T, Sprenger-Svačina A, Lin J, Ganesh BP, Lee J, McCullough LD, Sheikh KA, Zhang G (2024) Rejuvenating fecal microbiota transplant enhances peripheral nerve repair in aged mice by modulating endoneurial inflammation. Exp Neurol 376:114774. https://doi.org/10.1016/j.expneurol.2024.114774

Talley S, Valiauga R, Anderson L, Cannon AR, Choudhry MA, Campbell EM (2021) DSS-induced inflammation in the colon drives a proinflammatory signature in the brain that is ameliorated by prophylactic treatment with the S100A9 inhibitor paquinimod. J Neuroinflammation 18(1):263. https://doi.org/10.1186/s12974-021-02317-6

Tao X, Huang W, Pan L, Sheng L, Qin Y, Chen L, Yu L, Wu G, Wan J, Li H (2023) Optimizing ex vivo culture conditions to study human gut microbiome. ISME Commun 3(1). https://doi.org/10.1038/s43705-023-00245-5

Urushima H, Nishimura J, Mizushima T, Hayashi N, Maeda K, Ito T (2015) *Perilla frutescens* extract ameliorates DSS-induced colitis by suppressing proinflammatory cytokines and inducing anti-inflammatory cytokines. Am J Physiol Gastrointest Liver Physiol 308(1):G32–G41. https://doi.org/10.1152/ajpgi.00294.2014

Voetmann LM, Rolin B, Kirk RK, Pyke C, Hansen AK (2023) The intestinal permeability marker fitc-dextran 4kda should be dosed according to lean body mass in obese mice. Nutr Diabetes 13(1):1. https://doi.org/10.1038/s41387-022-00230-2

Weingarden A, González A, Vázquez-Baeza Y, Weiss S, Humphry G, Berg-Lyons D, Knights D, Unno T, Bobr A, Kang J, Khoruts A, Knight R, Sadowsky MJ (2015) Dynamic changes in short- and long-term bacterial composition following fecal microbiota transplantation for recurrent *Clostridium difficile* infection. Microbiome 3(1):10. https://doi.org/10.1186/s40168-015-0070-0

Wexler AG, Goodman AL (2017) An insider's perspective: *Bacteroides* as a window into the microbiome. Nat Microbiol 2(5):1–11. https://doi.org/10.1038/nmicrobiol.2017.26

Yousi F, Kainan C, Junnan Z, Chuanxing X, Lina F, Bangzhou Z, Jianlin R, Baishan F (2019) Evaluation of the effects of four media on human intestinal microbiota culture in vitro. AMB Express 9(1):69. https://doi.org/10.1186/s13568-019-0790-9

Yu EW, Gao L, Stastka P, Cheney MC, Mahabamunuge J, Torres Soto M, Ford CB, Bryant JA, Henn MR, Hohmann EL (2020) Fecal microbiota transplantation for the improvement of metabolism in obesity: the FMT-TRIM double-blind placebo-controlled pilot trial. PLoS Med 17(3):e1003051

Zhang W, Zou G, Li B, Du X, Sun Z, Sun Y, Jiang X (2020) Fecal microbiota transplantation (FMT) alleviates experimental colitis in mice by gut microbiota regulation. J Microbiol Biotechnol 30(8):1132