Fecal Microbiota Transplantation in Experimental Ulcerative Colitis Reveals Associated Gut Microbial and Host Metabolic Reprogramming

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ABSTRACT Fecal microbiota transplantation (FMT) is gaining attention for the treatment of ulcerative colitis (UC). Data from individual case studies have suggested that FMT may be beneficial for UC, but the detailed microbial and molecular basis remains unknown. Here, we employ 16S rRNA gene sequencing and metabolomics to investigate the influence of FMT on gut microbial community composition and host metabolism in the dextran sulfate sodium-induced UC rat model. The findings from this pilot study suggest that FMT from normal donors to UC recipients could alleviate UC symptoms without close resemblance of donor's gut microbial and metabolic pattern. Meanwhile, FMT from UC donors to normal recipient rats triggered UC symptoms, UC-prone microbial shift, and host metabolic adaption. Gut microbiota under normal conditions could maintain stable species richness and diversity upon FMT intervention, but the disturbed gut microbiota under UC conditions could not maintain such homeostasis. Significant correlations between altered bacterial composition and host metabolism could be assigned to the pathological effects of UC (accounting for 8.0 to 16.2% of total variance) and/or the FMT intervention effects (3.9 to 7.0% of total variance). Overall, our study reveals diverse gut microbial shifts in UC related FMT and their association with host metabolic reprogramming.

IMPORTANCE This study combined clinical symptoms measurement, 16S rRNA gene microbial profiling and metabolomics to comprehensively investigate the gut bacterial and host metabolic association and reprogramming in FMT-treated experimental UC. These data can advance our understanding of the effect of FMT on UC and the involvement of gut microbial dysbiosis in the development of UC.

KEYWORDS fecal microbiota transplantation, gut microbiota, host metabolism, ulcerative colitis

Fecal microbiota transplantation (FMT) aims to restore gut homeostasis by transferring feces from a healthy donor to a patient suffering from gut microbial imbalance. The therapy has been successfully used in the treatment of *Clostridium difficile* infection (CDI) (1–4). The success of treating CDI with FMT has promoted FMT-based therapeutics for other diseases, notably ulcerative colitis (UC), a chronic, relapsing, idiopathic, inflammatory disorder of the colon and rectum (5, 6). However, while CDI mainly results from *Clostridium difficile* infection and the resulting breakdown of gut microbiota diversity, the pathogenesis of UC is more complex, multifactorial, and not fully understood. Abnormal host immune responses, dysfunctional intestinal barriers, and gut microbiota dysbiosis, as well as environmental and other, unknown factors, are associated with UC (5, 6). Moreover, it is not yet known whether gut microbial dysbiosis is a cause or effect of UC.

Although individual case studies of two randomized controlled trials (7, 8) have

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suggested that FMT may be beneficial for UC, there were many inconsistencies between the results. In one study, 24% of patients who received FMT and 5% who received placebo entered remission after 7 weeks (7). In the other study, UC patients receiving FMT from healthy donors and autologous FMT (their own fecal microbiota) did not show statistically significant difference in clinical remission (8). Only 41% of patients who received donor FMT achieved clinical and endoscopic remission. A more recent study indicates that, despite variation in processes, FMT appears to be effective for inducing remission in UC, with no major short-term safety signals (9). Overall, there have been a limited number of studies evaluating the effectiveness of FMT in UC.

Numerous studies have shown that gut microbiota has a great impact on individual metabolite profiles (10–13). A recent study indicated that FMT restores normal fecal bile acid composition in recurrent CDI (1). However, integrated metabolomics and metagenomics have not been employed to study the effect of FMT in UC, the pathogenesis of which is more complex than CDI. Furthermore, it is unclear whether the metabolite alteration in FMT is due to the development of disease or microbial intervention. It is also unclear to what extent FMT changes the metabolic pattern. For metabolic profiling, urine offers many advantages over other biofluids due to its noninvasiveness, lack of volume limit, and less demanding sample preparation procedures for liquid chromatography-mass spectrometry (LC-MS) (14). In this study, we used targeted urinary metabolomics and high-throughput 16S rRNA gene sequencing to investigate the influence of FMT on the gut microflora and host metabolism in dextran sulfate sodium (DSS)-induced UC. We demonstrated that alteration of bacterial composition was accompanied by diverse and associated metabolic alterations.

RESULTS AND DISCUSSION

Normal-to-UC FMT alleviates UC symptoms without resembling the donor's gut microbial and metabolic pattern. In this study, we combined phenotyping, targeted metabolomics and 16S rRNA gene microbial profiling to comprehensively evaluate the impact of fecal microbiota transplantation in DSS-induced experimental UC. UC is characterized by periods of exacerbation (active disease) and remission (inactive disease). Most previous studies treated rats with 5% DSS for 7 days to establish experimental acute colitis (15, 16). Earlier studies in our laboratory found that colitis symptoms, such as rectal bleeding and diarrhea, occur as early as 2 to 3 days following DSS administration and that inflammation is fully installed within 6 to 7 days (17, 18). Thus, we carried out FMT from the initial stage (day 0) rather than after colitis establishment (day 7) to evaluate the impact of microbial changes to colitis development.

The unsupervised principal-component analysis (PCA) plot (Fig. 1) and clustered heatmap (see Fig. S1 in the supplemental material) showed significant intergroup distinction on days 3, 5, and 7 based on UC symptom indicators (Table 1). While the UC group showed a significantly increased disease activity index (DAI) score and pro- and anti-inflammatory cytokines, transplantation of fecal microbiota of normal rats effectively alleviated these UC symptoms in the UN group (i.e., UC recipients of normal fecal microbiome) on days 5 and 7 (see Fig. S1B and C in the supplemental material). Correspondingly, the UN group scattered closer to the N group in the PCA plot (Fig. 1B to D). It should be noted that the continued DSS treatment probably also affects the microbiota transplanted from the normal (N) group and thus obscures the influence of the N microbiota in the UN group. To minimize the influence of DSS on FMT, our future study will first establish the disease in the model and then perform FMT without DSS treatment to evaluate the impact of the N microbiota on UC.

The bacterial operational taxonomic unit (OTU) profile of the UC group on days 5 and 7 was essentially separated from that of other groups in the PCA plots (see Fig. S2A and C in the supplemental material). In contrast, the UN group clustered together with the N group, as well as normal recipients receiving FMT from normal donors (NN) (see Fig. S2A in the supplemental material), on day 5 and moved away from them on day 7 (see Fig. S2C in the supplemental material). Furthermore, the UN group showed the

FIG 1 PCA score plots of clinical UC symptoms in animal models on days 0 (A), 3 (B), 5 (C), and 7 (D). The percentage of total variance as explained by each component is shown in parentheses. Samples from different groups were represented by symbols of different color with 95% confidence ellipses. UC, colitis donor rats; N, normal donor rats; NU, normal rats receiving fecal suspension from colitis donors; NN, normal rats receiving fecal suspension from normal donors; UN, colitis rats receiving fecal suspension from normal donors.

most variable species richness, reduced on days 1 and 3 but increased on days 5 and 7 (see Fig. S3A in the supplemental material), suggesting FMT from normal donors to UC recipients finally reversed the trend of decreased species richness observed in UC. In contrast, normal recipients receiving FMT from normal or UC donors (NN or NU) did not change their gut bacterial species richness or diversity on days 3 to 7. These results suggest that gut microbiota under normal conditions could maintain stable species richness and diversity upon FMT intervention but that the disturbed gut microbiota under UC conditions could not maintain such homeostasis.

The PCA plots of rat urinary metabolome showed high intragroup variations and could not separate different groups from each other (see Fig. S2B and D in the

TABLE 1 Dynamic changes in body weight, disease activity index, and serum levels of cytokines of rats^a

Parameter	Day	Mean $\pm$ SD (n = 6)				
		N	UC	NN	NU	UN
Body wt (g)	0	360.8 $\pm$ 16.0	346.8 $\pm$ 19.0	350.3 $\pm$ 28.0	356.7 $\pm$ 22.2	364.7 $\pm$ 21.0
	3	359.7 $\pm$ 12.7	328.2 $\pm$ 18.0	343.8 $\pm$ 32.4	350.3 $\pm$ 27.7	351.0 $\pm$ 31.2
	5	364.2 $\pm$ 14.9	315.0 $\pm$ 24.7	344.0 $\pm$ 40.2	348.2 $\pm$ 29.2	351.2 $\pm$ 32.9
	7	365.2 $\pm$ 11.5	317.7 $\pm$ 22.9	347.0 $\pm$ 40.3	352.2 $\pm$ 32.2	353.2 $\pm$ 31.6
DAI	0	0	0	0	0	0
	3	0	1.3 $\pm$ 0.8	0.3 $\pm$ 0.5	0.7 $\pm$ 0.8	1.7 $\pm$ 0.5
	5	0	2.5 $\pm$ 0.8	0.3 $\pm$ 0.6	1.2 $\pm$ 0.8	1.5 $\pm$ 0.5
	7	0	2.8 $\pm$ 0.8	0.2 $\pm$ 0.4	0.7 $\pm$ 0.5	1.3 $\pm$ 0.5
Cytokine concn (pg/ml)						
IL-1 β	0	15.4 $\pm$ 7.9	17.5 $\pm$ 6.6	17.5 $\pm$ 5.5	17.8 $\pm$ 4.9	16.2 $\pm$ 6.0
	3	17.9 $\pm$ 4.2	41.4 $\pm$ 5.6	17.1 $\pm$ 3.6	27.0 $\pm$ 5.1	38.9 $\pm$ 6.2
	5	16.1 $\pm$ 2.2	86.8 $\pm$ 7.5	21.2 $\pm$ 4.4	32.8 $\pm$ 3.6	59.2 $\pm$ 9.0
	7	17.6 $\pm$ 3.4	103.4 $\pm$ 4.4	22.5 $\pm$ 4.4	35.6 $\pm$ 4.6	87.7 $\pm$ 5.7
IL-4	0	20.0 $\pm$ 3.7	22.0 $\pm$ 6.7	22.2 $\pm$ 4.3	21.6 $\pm$ 3.8	21.4 $\pm$ 3.7
	3	21.1 $\pm$ 3.0	65.5 $\pm$ 5.9	22.8 $\pm$ 5.2	29.7 $\pm$ 2.4	56.3 $\pm$ 10.1
	5	20.9 $\pm$ 3.4	105.3 $\pm$ 2.7	20.4 $\pm$ 6.8	52.8 $\pm$ 5.8	72.4 $\pm$ 8.0
	7	23.0 $\pm$ 5.1	141.9 $\pm$ 2.0	22.1 $\pm$ 5.3	64.3 $\pm$ 2.8	100.4 $\pm$ 5.6
IL-6	0	21.9 $\pm$ 5.1	22.9 $\pm$ 3.7	23.5 $\pm$ 5.1	23.5 $\pm$ 7.8	22.2 $\pm$ 5.3
	3	22.1 $\pm$ 5.0	107.0 $\pm$ 11.7	27.1 $\pm$ 6.9	53.2 $\pm$ 6.9	95.9 $\pm$ 7.7
	5	23.3 $\pm$ 4.8	134.4 $\pm$ 17.8	25.8 $\pm$ 6.9	68.1 $\pm$ 6.8	102 $\pm$ 17.8
	7	24.1 $\pm$ 4.1	174.7 $\pm$ 17.3	23.5 $\pm$ 7.2	82.2 $\pm$ 6.6	124.3 $\pm$ 14.5
IL-10	0	5.0 $\pm$ 6.9	6.2 $\pm$ 5.5	5.3 $\pm$ 2.0	5.1 $\pm$ 2.7	6.3 $\pm$ 3.2
	3	5.3 $\pm$ 2.5	51.3 $\pm$ 2.7	8.6 $\pm$ 5.8	27.3 $\pm$ 4.6	53.5 $\pm$ 5.7
	5	5.1 $\pm$ 3.0	71.0 $\pm$ 4.5	5.6 $\pm$ 2.1	37.2 $\pm$ 3.5	57.3 $\pm$ 3.2
	7	5.3 $\pm$ 2.7	104.1 $\pm$ 2.4	5.0 $\pm$ 1.5	46.9 $\pm$ 2.8	64.4 $\pm$ 5.9
TNF- α	0	21.8 $\pm$ 4.2	22.0 $\pm$ 3.4	21.3 $\pm$ 5.8	22 $\pm$ 2.6	21.9 $\pm$ 5.5
	3	22.1 $\pm$ 6.5	75.2 $\pm$ 9.7	22.3 $\pm$ 5.5	29 $\pm$ 6.1	67.1 $\pm$ 6.0
	5	22.4 $\pm$ 3.9	99.7 $\pm$ 8.2	23.1 $\pm$ 4.4	41.4 $\pm$ 2.8	86.8 $\pm$ 9.0
	7	23.0 $\pm$ 4.0	126.4 $\pm$ 8.4	23.9 $\pm$ 4.5	47.7 $\pm$ 4.4	100.3 $\pm$ 3.4

^aDAI, disease activity index. Blood samples (200 μ l each rat) were obtained from orbital sinuses on days 0, 3, 5, and 7. Cytokines were assayed by ELISA.

supplemental material). To further increase the group distinction, supervised partial least-squares discriminant analysis (PLS-DA) was used, which revealed that, compared to the UC group, the UN group diverged more significantly from the N and NN groups at both species and genus levels (Fig. 2A, B, D, E, G, and H). Taken together, our study suggested that FMT contributed UC remission could be achieved through alternative microbial and host metabolic reprogramming in the recipients without close resemblance of donor's gut microbial and metabolic pattern. Hippuric acid, a dominant host-microbial cometabolite, has been reported to be reduced under UC and Crohn's disease conditions due to differential gut microbial metabolism (19, 20, 21). Hippuric acid was significantly reduced in the UC and UN groups from days 2 to 5 and from days 1 to 7 (except day 6), respectively (Fig. 3A). The extended duration of hippuric acid reduction in the UN group suggested a more sensitive (since it changed on day 1) and pronounced UC-prone metabolic shift. The relative abundances of C_{10:3} acylcarnitine, hydroxyphenylpropionylglycine, and riboflavin remained virtually unchanged among the N, NN, and NU groups (Fig. 3B to D). The levels increased in UC group and increased even more dramatically in the UN group in most cases. Acylcarnitines and acylglycines are important biochemical markers for the diagnosis of disorders of mitochondrial fatty acid β -oxidation (22, 23, 24), and their increase signified disturbances in fatty acid β -oxidation in the UC and UN groups. It should be noted that unlike medium-chain acylcarnitines (e.g., C_{10:3} acylcarnitine), short-chain acylcarnitines such as acetylcarnitine, butyrylcarnitine, and C-5=O acylcarnitine did not show significant change. It should also be noted that the high sensitivity and metabolite coverage of LC-MS revealed more metabolic alterations in UC than noted in a previous nuclear magnetic resonance (NMR)-based study (20, 25).

Two highly positively correlated ($R = 0.67$, $P < 0.000001$) *cis*-3,4-methylene derivatives (*cis*-3,4-methylene-heptanoylcarnitine and *cis*-3,4-methylene-heptanoylglycine)

FIG 2 PLS-DA score plots of gut bacterial composition at the species (A, D, and G) and genus (B, E, and H) levels, as well as host urinary metabolome profiles (C, F, and I), on days 3, 5, and 7. The percentage of total variance as explained by each component is shown in parentheses. Samples from different groups are represented by symbols of different color with 95% confidence ellipses. The leave-one-out cross-validation (LOOCV) results for each PLS-DA model are as follows: A ($R^2 = 0.84$, $Q^2 = 0.43$), B ($R^2 = 0.74$, $Q^2 = 0.58$), C ($R^2 = 0.97$, $Q^2 = 0.65$), D ($R^2 = 0.70$, $Q^2 = 0.34$), E ($R^2 = 0.74$, $Q^2 = 0.43$), F ($R^2 = 0.91$, $Q^2 = 0.58$), G ($R^2 = 0.62$, $Q^2 = 0.18$), H ($R^2 = 0.82$, $Q^2 = 0.55$), and I ($R^2 = 0.91$, $Q^2 = 0.57$).

were also increased in the UN and UC groups but remained constant in the other three groups (see Fig. S4 in the supplemental material). Once more, the extended duration of alteration was observed in the UN group. Libert et al. reported that *cis*-3,4-methylene-heptanoylcarnitine is present in human urine, except in urine from newborn patients (26). These researchers further found that the acylcarnitines with a cyclopropane ring in their fatty acid moieties disappeared in the urine of humans treated with antibiotic. Fatty acids with cyclopropane rings can be synthesized in bacterial membranes by

FIG 3 Increased UC-prone metabolic shift in UC rats receiving FMT from normal rats (UN). Data are expressed as box-and-whisker plots with median, quartiles, and ranges. The relative abundances of hippuric acid (A), $C_{10:3}$ acylcarnitine (B), hydroxyphenylpropionylglycine (C), and riboflavin (D) are presented. *, $P < 0.05$ versus N; **, $P < 0.01$ versus N; #, $P < 0.05$ (UN versus UC).

cyclopropane fatty acid synthase (27). Therefore, it has been proposed that *cis*-11,12-methylene-pentadecanoic acid generated by cyclopropane fatty acid synthase in bacteria could be absorbed and metabolized through β -oxidation in mitochondria to form *cis*-3,4-methylene-heptanoyl coenzyme A, which would then be converted into acylcarnitines and excreted (28). Therefore, the increased *cis*-3,4-methylene derivatives may also be due to the altered host utilization of bacterial fatty acids.

UC-to-normal FMT triggers UC symptoms, microbial shift, and host metabolic adaption. FMT from UC to normal rats (NU) resulted in UC symptoms, including an increased DAI score and pro- and anti-inflammatory cytokine concentrations (Table 1 and Fig. S1 in the supplemental material) and thus shifted to the UC group in PCA plots (Fig. 1). PLS-DA plots illustrates the gradual microbial shift from normal to UC status at species (Fig. 2A, D, and G) and genus (Fig. 2B, E, and H) levels from days 3 to 7. There was also a subtle alteration of NU metabolome, which partially shifted to the UC zone on days 3 and 5 and shifted back to N and NN on day 7 (Fig. 2C, F, and I). Hippuric acid showed a tendency toward reduction in the NU group on day 3 ($P = 0.06$), was significantly reduced on day 4 ($P < 0.05$) and day 5 ($P < 0.01$), and was restored to

FIG 4 Number of significant correlations between the top 25 discriminative OTUs and the top 25 discriminative metabolites on days 1, 3, 5, and 7.

normal levels on days 6 and 7 (Fig. 3A). The restoration on days 6 and 7 was probably due to the ongoing host metabolic adaptation to FMT intervention. In contrast to the UN group, FMT from normal to normal (NN) did not introduce appreciable alteration in gut bacterial or host metabolic profiles, proving that the fecal microbial composition of FMT donors has a significant effect on recipients. It should be noted that in this study, FMT was performed by a single oral dose of a 1-g/kg fecal suspension for 7 days. Thus, a 250- to 350-g rat will only receive 1.5 to 2.1 mg of DSS per day through FMT based on a previous report showing that only 6 mg of DSS existed in 1 g of feces from a DSS-treated rat (29). In contrast, a rat would receive 2.5 to 4.0 g of DSS per day through drinking water (50 to 80 ml of water containing 5% DSS). Given that FMT introduced 1,700- to 2,700-fold less DSS than drinking water, the fecal DSS would have negligible effect on rats. A previous study showed that the transplantation of multiple enteric bacterial species (*Bacteroides* species, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas fluorescens*, and *Fusobacterium varium*) and *Mycobacterium paratuberculosis* to germfree mice results in the development of chronic intestinal inflammation (30). In addition, germfree mice colonized by gut microbiota from UC patients were more susceptible to colitis than mice receiving microbiota from healthy humans (31). In accordance with previous reports, our study indicates the involvement of gut microbiota in the pathogenesis of UC.

UC-related associations between gut microbial and host metabolic shifts. To decipher whether there were associations between gut bacterial and host metabolic alterations in the development of UC, we performed correlation analysis of the top 25 OTUs and metabolites based on PLS-DA variable importance in projection (VIP) scores. A total of 16, 21, 36, and 43 significant correlations ($P < 0.01$) between the top 25 OTUs and metabolites were observed on days 1, 3, 5, and 7, respectively (Fig. 4 and see Fig. S5 to S8 in the supplemental material), suggesting that gut microbial alterations play an increasingly important role in host metabolic shift during UC development. A characteristic association was observed on day 7 featuring three amino acids [L-tyrosine, L-methionine, and L-(iso)leucine] simultaneously associated with the same four OTUs (two OTUs were assigned to the genus *Oscillospira*, one to the family *Ruminococcaceae*, and one to the order *Clostridiales*) (see Fig. S8 in the supplemental material). Phenyl derivatives hydroxyhippuric acid and hexanoylglycine were constantly correlated with certain OTUs. Specifically, hydroxyhippuric acid was associated with the families *Rikenellaceae* and S24-7, the genera *Ruminococcus* and *Oscillospira*, and the species *Ruminococcus gnavus* and *Bacteroides acidifaciens*; hydroxyphenylacetyl glycine was associated with the families *Ruminococcaceae* and *Enterobacteriaceae*, the genus *Oscillospira*, and the species *Ruminococcus gnavus*. Furthermore, most metabolites that correlated with certain OTUs such as benzoyl, and phenyl derivatives, indole derivatives, and polyamines have been demonstrated to be host-microbial cometabolites which are products of combinatorial metabolism of substrates by gut microbiome and host (32). However, these cometabolites did not show constant correlation with some

FIG 5 (A) Hierarchical clustering heatmap at the genus level. Family level (f_) assignments were provided if the genus (g_) level resolution was not achieved. (B) FMT-triggered bacterium-metabolite correlations in the UN group on day 7. Positive and negative correlations with a significance level of $P < 0.01$ are highlighted in red and blue, respectively.

specific OTUs, probably because they were produced by multiple bacterial species and were also affected by host factors.

FMT-related associations between gut microbial and host metabolic shifts. To further investigate FMT-related association, we selected bacteria significantly increased in FMT recipient groups (e.g., NN, NU, and UN) for correlation analysis as they were more likely to introduce host metabolic alteration. In this study, we focused on nine genera (*Anaerostipes*, *Bacillus*, *Carnobacterium*, *Dehalobacterium*, *Dorea*, *Geobacillus*, *Lactococcus*, *Oscillospira*, and *Rhodanobacter*) and three families (*Bacillaceae*, *Exiguobacteraceae*, and *Bradyrhizobiaceae*) that were dramatically increased in the UN group on day 7 (Fig. 5A). They accounted for the growing microbial divergence of the UN group (see Fig. S3B in the supplemental material). Among the selected nine genera and three families, four genera (*Anaerostipes*, *Rhodanobacter*, *Carnobacterium*, and *Geobacillus*) did not exhibit significant correlation with measured metabolites. The genera *Oscillospira* and *Dehalobacterium* and the families *Bacillaceae* and *Exiguobacteraceae* contributed to the majority of correlations (Fig. 5B). Compared to the above UC-related gut bacterium-host metabolic association, two metabolites, riboflavin and trimethylguanosine ($P < 0.01$), were unique in FMT-triggered gut bacterium-host metabolic association. Interestingly, all nucleotide-related metabolites, including guanine, trimethylguanosine, and acetylcytidine, were negatively correlated with certain bacteria: genus *Lactococcus*, family *Exiguobacteraceae*, and genus *Oscillospira*, family *Bacillaceae*, respectively.

FMT intervention effect is weaker than the UC pathological effect. As shown in Fig. 2, the first principal component (PC1) of PLS-DA was mainly UC related: most normal donors and recipients (N, NN, and NU) had a negative score, whereas most UC donors and recipients (UC and UN) had a positive score. By analyzing the top eight PCs, we further found several FMT-related PCs, namely, PC7 of day 5 OTUs, PC5 of day 7 OTUs, and PC6 of day 5 metabolome. As shown in Fig. S9 in the supplemental material, in the FMT-related PC direction, most UC recipients of normal fecal microbiome (UN) clustered with the normal group (N), and most normal recipients of UC fecal microbiome (NU) clustered with UC. However, samples were simply randomly distributed in other FMT-nonrelated PC directions (data not shown). They accounted for only 4.2, 7.0, and 3.9% of the total OTU variation, respectively (see Fig. S9 in the supplemental material). In contrast, the corresponding percentages of explained variance by UC-

related PC1 were 8.0, 16.2, and 16.9% (Fig. 2A, D, and G), which were 1.9-, 2.3-, and 4.4-fold greater than their FMT-related counterparts, respectively, suggesting that the FMT intervention effect was weaker than the UC pathological effect in microbial and metabolic reprogramming. Similarly, a recent study found that FMT from highly feed-efficient donors shows little effects on age-related changes in feed efficiency-associated fecal microbiota in chickens (33). Taken together, these results suggest that host- and environment-related factors may more strongly affect host fecal microbiota and metabolism than the FMT.

MATERIALS AND METHODS

Animals and FMT. Male Sprague-Dawley rats (250 to 300 g; Animal Facility of University of Macau) were housed in a temperature-controlled room ($23 \pm 1^\circ\text{C}$) with a 12-h light/dark cycle under specific-pathogen-free conditions. Rats were allowed to acclimate to the new environment for 4 days prior to experiments with *ad libitum* access to standard chow (comprised of corn, fish meal, wheat flour, salt, vitamins, trace elements, amino acids, etc.) and demineralized water. All experimental procedures involving animals were approved by the Animal Research Ethics Committee, University of Macau (UMAEC-2015-09).

Rats were randomly divided into five groups ($n = 6$ each group). Two donor groups were colitis donor rats (UC) and normal donor rats (N). Three recipient groups were normal rats receiving fecal suspension from colitis (NU) and normal (NN) donors and colitis rats receiving fecal suspension from normal donors (UN). Throughout the experimental period, rats were fed standard chow, and bottles were refilled daily with fresh 5% DSS (MP Biomedicals, Santa Cruz, CA) for the UC and UN groups or demineralized water for the N, NN, and NU groups for 7 days. The fecal samples from normal and UC donor rats were freshly collected daily at 10:00 a.m. and pooled at equal amounts within each group. One gram of pooled fecal samples from each donor group was suspended in 10 ml of sterile 0.9% normal saline by vortexing. The FMT was performed by a single oral administration of a 1-g/kg fecal suspension for 7 days. Body weight and stool consistency of each rat were recorded daily. The blood samples were collected on days 0, 3, 5, and 7. Fecal microbiota and urine samples were obtained by taking individual rats out of their cages and collecting fecal pellets and drops of urine. Fecal and urine samples were collected on days 0 to 7 and stored at -80°C immediately after collection.

Assessment of clinical symptoms in animal models. Each rat was given a disease activity index (DAI) score based on weight loss, stool consistency, and bloody stool according to our previous report (34). Blood samples (200 μl) were obtained from the orbital sinus, and proinflammatory (interleukin-1 β [IL-1 β], IL-6, and tumor necrosis factor alpha [TNF- α]) and anti-inflammatory (IL-4 and IL-10) cytokines in the serum were measured using enzyme-linked immunosorbent assay (ELISA) kits from Excell Biological Co., Ltd. (Shanghai, China), according to the manufacturer's instructions.

Targeted urinary metabolomics. Portions (25 μl) of urine were mixed with 100 μl of 5% methanol, vortexed, and centrifuged at $14,000 \times g$ for 15 min (4°C). The supernatant was subjected to targeted urinary metabolomics analysis with an Agilent 1200 rapid-resolution liquid chromatograph coupled with a 4000 QTRAP (Applied Biosystems/MDS Sciex) mass spectrometer using the same parameters as in our previous study (14). Briefly, 5 μl of sample was separated on a Zorbax SB-C₁₈ (100 by 2.1 mm, 1.8 μm ; Agilent) column at the flow rate of 200 $\mu\text{l min}^{-1}$ using 0.1% formic acid in H₂O (A) and 0.1% formic acid in acetonitrile (B). The mass spectrometer was operated in positive-ion mode, and scheduled multiple reaction monitoring was used for targeted quantification of 410 transitions covering 235 identified metabolites with the same collision energy and declustering potential listed in Table S2 in our previous study (14). The quantified metabolites included amino acids, acylglycines, acylcarnitines, bile acids, cholines, indoles, nucleotides, polyamines, purines, dipeptides, and steroids, which was comprehensive enough to provide important biological information. The scan time and the detection window were 2 and 140 s, respectively. Following acquisition, data were extracted by peak finding and alignment using the MarkerView software 1.2 (AB Sciex) with the following parameters: smoothing half-width, 1 point; baseline subtraction window, 2.0 min; noise percentage, 50%; peak-splitting factor, 2; minimum required intensity, 300; minimum peak width, 6 points; minimum signal-to-noise, 5.0; maximum number of peaks, 1,000; and retention time tolerance, 0.2 min.

Microbial community profiling by 16S rRNA gene sequencing. Microbial genomic DNA was extracted from fecal samples by using a Qiagen QIAamp DNA stool minikit according to the manufacturer's protocol. PCR was performed using the primers 338F (5'-ACTCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (95°C for 3 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and 72°C for 10 min) (35). Microbial community composition was determined by sequencing the V3-V4 variable region of the 16S rRNA gene using the Illumina MiSeq platform (San Diego, CA) and trimmed using a 5-bp sliding window with 1-bp-length steps based on the phred algorithm (36). The sequenced reads were assembled into single end reads using FLASH version 1.2.6 (37). Chimeric sequences were removed using UCHIME (38) in mothur version 1.31.2 (39), and sequences that exhibited the following characteristics were discarded: read length, <200 bp; ambiguous base calling; six-base homopolymer runs; lack of primers; primer mismatches; or uncorrectable barcodes. The 16S rRNA sequences were clustered into species-level phylotypes using the closed-reference OTU picking protocol (40) against Greengenes database version 13.5 (41) with a threshold of 97% using the Quantitative Insights into Microbial Ecology (QIIME) software (42). The most abundant read from each

OTU was selected as the representative read for that OTU. The taxonomy associated with the Greengenes database to which OTUs matched was assigned to OTUs.

Multivariate analysis. Microbiome data, as well as the metabolomics data, were subjected to data processing, normalization, scaling, and multivariate analyses using MetaboAnalyst 3.0 (43). Features with at least 50% missing values were removed by using a data integrity check. The remaining missing values were replaced with a small value (the half of the minimum positive values in the original data). Then, data were filtered based on the interquartile range to remove baseline noises that are unlikely to be of use when modeling the data. Data were further normalized to total intensity followed by Pareto scaling to obtain normally distributed variables. PCA and PLS-DA were performed to identify distinct OTUs and metabolite patterns. Discriminative OTUs and metabolites were selected based on the VIP score generated from PLS-DA. Pearson's correlation analysis was carried out using SPSS Statistics 20 (IBM, New York, NY), and significance levels were established at $P < 0.05$. The significance of the differences between groups was determined by a two-tailed Student *t* test using GraphPad Prism software. A Mann-Whitney nonparametric test was used for metabolite data graphed as box-and-whisker plots with medians, quartiles, and ranges.

Accession number(s). Raw sequencing data are available in the NCBI SRA BioProject database under accession no. [SRP132248](https://www.ncbi.nlm.nih.gov/sra/SRP132248).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AEM.00434-18>.

SUPPLEMENTAL FILE 1, PDF file, 1.5 MB.

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R.Y., Z.-X.Y., and X.-J.G. conceived and designed the study. Z.-X.Y., X.-J.G., T.L., B.W., P.-P.W., and Y.Y. conducted the animal study and collected samples. Z.-X.Y. and X.-J.G. performed data analysis. Z.-X.Y. and R.Y. wrote and edited the manuscript. All authors read and approved the final version of the manuscript.

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