Effect of Massa Medicata Fermentata on the Gut Microbiota of Dyspepsia Mice Based on 16s rRNA Technique

Xiaorui Zhang  
Chengdu University of Traditional Chinese Medicine

Hongling Zhang  
Chengdu University of Traditional Chinese Medicine

Qinwan Huang  
Chengdu University of Traditional Chinese Medicine

Jilin Sun  
Sichaun Fuzheng Pharmaceutical Co. Ltd

Renchuan Yao  
Sichuan Fermentation Traditional Chinese Medicine Engineering Research Center

Jin Wang  
Chengdu University of Traditional Chinese Medicine

Research

Keywords: Massa MedicataFermentata, Chinese fermentation medicine,dyspepsia mice, functional dyspepsia, intestinal microorganisms, 16s rRNA gene high-throughput sequencing

Posted Date: June 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-31452/v1

License: ©  This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License

Version of Record: A version of this preprint was published at Evidence-Based Complementary and Alternative Medicine on September 23rd, 2020. See the published version at https://doi.org/10.1155/2020/7643528.
Abstract

Background: Massa Medicata Fermentata (MMF), also known as Shenqu and Liuqu, is a traditional Chinese medicine (TCM) for treating indigestion and its related disorders. This study analyzes the effect of MMF on intestinal microorganisms in dyspepsia mice based on 16S rRNA technology.

Methods: We adopt a dyspepsia model of spleen deficiency, which is caused by a high-protein, high-calorie, high-fat diet. The 60 specific-pathogen free Kunming (SPF KM) mice were randomly divided into a model group (administered saline 50 ml/kg/day, n=12), an MMF group (LSQ group, administered 4.8 g/kg/day, n=12), a Jianweixiaoshi group (JWXS group, administered 2.88 g/kg/day, n=12), a Domperidone group (DP group, administered 0.006 g/kg/day, n=12), and a blank group (administered saline 50 ml/kg/day, n=12). On the seventh day of administration, the mice were fasted and deprived of water. After 24 h, take the second feces of stress defeation in mice under strict aseptic conditions and quickly transfers them to a sterile cryotube. This study comprehensively evaluates the α-diversity, β-diversity, flora abundance and composition of each group of mice's intestinal microorganisms, and their correlation with functional dyspepsia on the base of 16S rRNA gene sequencing technology.

Results: After modeling, there were some dyspepsia reactions, proximal gastric relaxation reduction, and changes in intestinal microflora. Dyspepsia mice appeared dyspepsia reactions and proximal gastric relaxation reduction, characterized by a significant decrease of contents of gastrin (P <0.01) and cholinesterase (P <0.01). MMF can improve dyspepsia symptoms and promote proximal gastric relaxation. Significant intestinal flora disorders were observed in dyspepsia mice, including down-regulation of Bacteroidetes, Lactobacillus, and Prevotellaceae, up-regulation of Proteobacteria, Verrucomicrobia, Epsilonbacteraeota, Firmicutes, Lachnospiraceae NK4A136 group, and Lachnospiraceae. MMF could alleviate intestinal microflora disturbance, and the regulation effect of MMF on Bacteroidetes, Verrucomicrobia, and Epsilonbacteraeota was more reliable than that of Jianweixiaoshi tables and Domperidone. The intestinal microflora may be correlated with the promote digestion of MMF.

Conclusions: Overall this research explained the potential pharmacological mechanism of MMF and provided targets and direction for further research on Chinese fermentation medicine. Such research-based on high-throughput data sets can be used to interpret TCM theories and provide valuable research models and clinical medication references for TCM researchers and doctors.

Background

Dyspepsia, also known as functional dyspepsia (FD), is a common functional gastrointestinal disorder (FGID), mainly characterized by postprandial fullness, early satiety, upper abdominal pain, upper abdomen burning, belching, nausea and other dyspeptic symptoms [1]. The incidence of FD is high, 11%~20% in Western countries, and 11.8%~23.8% in China [2,3]. Most patients have chronic, recurrent symptoms that affect their quality of life to varying degrees. The pathogenesis has not yet been fully elucidated, and most of them are considered to be associated with abnormal gastrointestinal motility and high visceral sensitivity [4]. Small intestinal bacterial overgrowth (SIBO) can manifest various digestive and malabsorption manifestations, such as abdominal pain, bloating, diarrhea, weight loss, etc. [5]. Gastric motility disorder can cause food siltation and bacterial growth in the upper digestive tract, leading to SIBO [6]. Therefore, gastrointestinal motility abnormalities may be related to the occurrence of SIBO in patients with FD, and its potential clinical significance is worthy of attention. Many FD symptoms, and their refractory features mainly include complex dysmotility...
and visceral hypersensitivity. In addition to the high tension of the cerebral cortex, they are also involved in changes in intestinal microecology, imbalance of mediation mechanisms of the gastrointestinal mucosal immune-inflammatory response. Besides, the combined use of probiotics is conducive to the improvement of dyspeptic symptoms, suggesting that intestinal flora disorders may be involved in the pathogenesis of FD, which also provides new ideas for the treatment of FD.

*Massa MedicataFermentata* (MMF), also known as *Shenqu* and *Liuqu*, is a traditional Chinese medicine for treating indigestion and its related disorders. The main functions of MMF are related to the protection of the spleen and stomach and promotion of digestion, which is especially suitable for children's functional dyspepsia in clinical practice. MMF is made up of a certain proportion of flour, wheat bran, rice bean powder, and bitter apricot seed powder. And then added with water extract of *Artemisia centifolia*, *Polygonum hydropiper L.* and *Xanthium sibiricum*, to make a mixture of consistency humidity, the ratio of the above raw materials used is in turn 25:50:1:1:5:5:5, and then pressed into a small square with a fixed mold. It is fermented under a constant temperature of 36°C and a humidity of 75%.

Our previous research confirmed that MMF protects the spleen and stomach and promotes digestion functions, but we did not analyze the specific flora. Therefore, we used 16s rRNA sequencing to study the corresponding changes in the intestinal flora to understand the mechanism of action of the MMF district.

**Materials And Methods**

**Animals**

A total of 30 male SPF KM mice and 30 female SPF KM mice (18-22 g) were obtained from Chengdu Dashuo Biotechnology Co., Ltd. (Chengdu, China). According to the Declaration of Helsinki, all animals received humane care, which was promulgated in 1964 and amended in 1996. All experimental protocols were approved by the Animal Care and Use Committee of Chengdu Municipal Hospital of Traditional Chinese Medicine, Chengdu, China (approval no. SCXK (Chuan) 2015-30). The animals were kept in a sterile animal room, which includes individually ventilated cages, and the feed and drinking water were subjected to autoclaving during the test.

**Modeling and treatment**

After adaptation to feeding, 12 mice were randomly selected as a blank group, and the remaining mice were made into a dyspepsia model. A blank group was given a regular diet, and the other groups were fed a self-made high-protein, high-calorie diet (from soy flour, fish pine, flour, milk powder in a ratio of 2:1:1:1) and thoroughly mixed with water. It is made into a biscuit shape and dried. At the same time, feed 50% milk 2 mL·(kg)-1, free feeding, and drinking for 7 days, causing the spleen deficiency model. The body weight, abdominal circumference, and average food intake and feces of each mouse were measured and recorded on days 2, 5, and 8. Compared with the blank group, food intake and feces were significantly reduced, abdominal fullness and abdominal circumference increased, and the spleen deficiency food was successfully simulated. After successful modeling, the 48 mice were randomly divided into a model group (administered saline 50 ml/kg/day, n=12), an MMF group (LSQ group, administered 4.8 g/kg/day, n=12), a Jianweixiaoshi group (JWXS group, administered 2.88 g/kg/day, n=12), a Domperidone group (DP group, administered 0.006 g/kg/day, n=12), and a blank group (administered saline 50
Sample collection

On the seventh day of administration, the mice were fasted and deprived of water. After 24 h, take the second feces of stress defecation in mice under strict aseptic conditions and quickly transfer them to a sterile cryotube. The cryotubes were stored in a -80°C refrigerator for later use. Before the mice were sacrificed, blood was taken from the eyeballs. Then centrifuged at 3000 rpm for 15 min, and the serum was collected and stored in the refrigerator at -70 °C for later use. Follow the instructions of the three kits to determine the contents of gastrin, cholinesterase, and nitric oxide in serum.

Determination of gastrin, cholinesterase and nitric oxide in serum

Take frozen mouse sera and test gastrin, cholinesterase, and nitric oxide according to the instructions.

DNA extraction and purification

The mice fecal bacterial genome total DNA was extracted using a soil DNA kit according to the kit standard according to the manufacturer's instructions, and the extracted genomic DNA was detected using 2% agarose gel electrophoresis and a super differential spectrophotometer (Thermo Fisher). The purified DNA extract was stored at -80 °C until use.

MetaVxTM Library Preparation and Illumina MiSeq Sequencing

Take the feces of the mouse, extract the DNA, and use 0.8% agarose gel electrophoresis to detect the DNA. The purified genomic DNA was amplified by polymerase chain reaction (PCR) according to experimental instructions. 515F (5'-GTGYTACMGCGGCGGTV∥-3) and 806R (5'-GGACTACHVGGGTWTCTV∥T-3) were used as primers [11,12]. Perform PCR product detection, purification, and quantification on the test sample. Use the TruSeq DNA PCR-Free Sample Preparation Kit to construct the library. After the quantified library has been quantified and the library is qualified, it is sequenced using the PE250 mode of the MiSeq 3000 platform PE300 of Chengdu Luoning Biotechnology Co., Ltd. The original offline data obtained by sequencing is spliced. Filter to get the high-quality target sequence required for subsequent analysis.

The library was constructed using the TruSeq DNA PCR-Free Sample Prep Kit (Illumina, FC-121-3001/3003). After the constructed library was qualified and tested by the library, it was sequenced using the MiSeq 3000 platform PE300 mode sequencing, sequencing kit. Use the Hiseq Rapid SBS Kit v2 (Illumina, FC-402-4023 500 Cycle). DNA samples were quantified using a Qubit 2.0 Fluorometer (Thermo Scientific). V4 hypervariable regions of microbial 16S rDNA were selected for generating amplicons and following taxonomy analysis. Synthesis of specific primers with Barcode for the 16S rRNA gene in fecal DNA extracts PCR amplification, the corresponding sequences of primers are 515F (5'-GTGYTACMGCGGCGGTV∥-3) and 806R (5'-GGACTACHVGGGTWTCTV∥T-3), respectively. Each 25 μL system included 1x PCR buffer, 1.5 mM MgCl₂, 0.4 μM dNTPs forward and reversed primers of 1.0 μM each, 0.5 U KOD-Plus-Neo enzyme (TOYOBO) and 10 ng template. The PCR procedure consisted of starting at 94 °C for 1 min and then 30 cycles (denaturation at 94 °C for 20 s, annealing at 54°C for 30 s and 2 °C for 5 min. Three replicates were performed for each sample. After
the end of the PCR, the PCR products of all the same samples were mixed and subjected to electrophoresis detection. A recovery kit recovered the PCR products, and the target DNA fragment was eluted with TE buffer. PCR was mixed with 1/6 volume of 6×loading buffer and detected by agarose gel electrophoresis. Take the strip for recycling and recycle the QIAquick Gel Extraction Kit (QIAGEN).

Data analysis

Based on Usearch (http://drive5.com/uparse/) software, OTUSE algorithm[^13] is used to perform OTU clustering at 97% consistency level, and the highest frequency sequence in each OTU is selected as the representative one. Annotated analysis was performed using the UCLUST classification[^14] and the SILVA database (Release_123 http://www.arb-silva.de/). Representative sequences were subjected to multiple alignments using PyNAST. Use FastTree[^15] to build a phylogenetic tree. Each sample was homogenized and resampled, with the least amount of data in the sample. Community composition analysis was performed using the R language[^16] for various data transformations. Use the ggplot[^17] package to the plot. Differential species analysis uses the Python LEfSe package. Random forest analysis uses the R language randomForest package. The metastatic analysis is performed using the R language. Correlation analysis uses the cor.test function of R’s stats package. Use the R language psych and corrplot package to analyze the relationship between the promotion of digestion and decreased gastric relaxation and bacteria. The analysis results were statistically significant, with P<0.05. Data were expressed as mean ± standard deviation (M ± S), basic statistical analysis and charting were performed using SPSS Statistics v17.0 statistical analysis software, one-way ANOVA, and LSD and Dunnett’s T3 method were used. After the two-two comparative analysis, those who do not conform to the normal distribution use the rank-sum test. P < 0.05 was considered statistically significant.

Results

Effects on serum gastrin, cholinesterase, and nitric oxide

It can be seen from Table that compared with the blank group, the gastrin, and cholinesterase in the model group were significantly reduced (P <0.01), and nitric oxide was significantly increased (P <0.01). Compared with the model group, the administration groups can significantly increase the levels of gastrin and cholinesterase (P <0.01, P <0.05), and can also significantly reduce the level of nitric oxide (P <0.01, P <0.05).
### Table 1

| Groups      | Dose/(g·kg⁻¹) | Gastrin(μg/mL) | Cholinesterase(nmol/l) | Nitric oxide(μmol/l) |
|-------------|---------------|---------------|------------------------|----------------------|
| Blank group | -             | 2.54±0.31     | 152.69±4.08            | 57.75±4.02           |
| Model group | -             | 1.85±0.18**   | 132.24±5.20**          | 88.12±4.57**         |
| LSQ group   | 4.8           | 2.40±0.29##   | 145.55±2.82#           | 77.67±3.97#          |
| JWXS group  | 2.88          | 2.45±0.35#    | 144.54±3.14#           | 76.61±3.36#          |
| DP group    | 0.006         | 2.23±0.29#    | 148.05±2.76##          | 71.20±2.78##         |

Compare with the blank group** means P<0.05, ** means P<0.01
Compare with the model group## means P<0.05, # means P<0.01

### Analysis of the diversity of intestinal flora in mouse

By analyzing the sample dilution curve, Rank-Abundance curve, and alpha diversity index to study the richness, uniformity, and diversity of the mouse intestinal flora, see Table and figure 1, 2. The large dilution curve and rank-abundance curve indicate that the sequencing depth is sufficient and can cover most species (all sample coverage is higher than 0.99). There are differences between the chao1, Shannon, and PD indexes of the model group and the blank group (P<0.05), and there are also significant differences between the Shannon and Simpson indexes of the administration groups and the model group (P<0.01). The PD index of JWXS group was significantly different from that of the model group (P<0.01), which indicated that the treatment of the three drugs could promote the recovery of the diversity of intestinal flora in mice with food accumulation.

### Table 2

| Groups      | OTU       | Chao1       | Shannon | Simpson | Coverage | PD         |
|-------------|-----------|-------------|---------|---------|----------|------------|
| Blank group | 852.17±12.31 | 1048.24±31.09 | 4.79±0.01 | 0.98±0.0006 | 0.99 | 67.71±0.76 |
| Model group | 993.83±11.00** | 1220.22±29.33* | 5.02±0.01** | 0.98±0.0002 | 0.99 | 75.65±0.96** |
| LSQ group   | 1187.17±79.03 | 1502.37±124.59 | 5.44±0.04## | 0.99±0.0005## | 0.99 | 89.37±6.21 |
| JWXS group  | 886.83±12.53## | 1083.76±25.53# | 4.91±0.01## | 0.98±0.0017 | 0.99 | 69.38±0.91## |
| DP group    | 926.83±9.56## | 1138.82±12.08 | 4.91±0.013## | 0.98±0.0008 | 0.99 | 72.36±0.94 |

Compare with the blank group** means P<0.05, ** means P<0.01
Compare with the model group## means P<0.05, # means P<0.01
The principal coordinate analysis (PCoA) is shown in figure 3, and it is found that the percentages explained by PC01 and PC02 to the overall variance are 67.4% and 17.8%, respectively. The high-dose LSQ group is very close to the JWXS tablet group, and completely separated from the DP group, and the blank group is wholly separated from the model group. It shows that the intestinal flora of mice has changed significantly in the state of food accumulation, and MMF, Jianweixiaoshi tables, and Domperidone have a tendency to reverse this change.

To intuitively compare the similarity between different samples, cluster analysis is used for calculation and graphing. Based on different distance matrix for cluster analysis, the results are shown in figure 4. The closer the sample is in the figure, the shorter the branch length is, indicating that the two samples' community structure is similar. The results showed that the distance between the model group and the blank group was the longest, and the distance between the LSQ group and the blank group was between the two control groups, indicating that the internal flora structure was close to the blank group after administration. The blank group is the farthest from the model group, and the closest to the blank group is the JWXS group, the LSQ group, and the DP group.

Analysis of the structural composition of intestinal flora in mice

We can understand the annotation status of OTU at the phylum and genus levels in the intestinal flora of mice by analyzing the microflora in the feces of accumulating mice.

At the door level, the five groups are mainly Bacteroidetes (32.03% to 72.60%), Firmicutes (18.44% to 48.82%), Proteobacteria (3.18% to 7.86%) and Epsilonbacteraeota (0.67% to 5.83%). Various types of bacteria and their proportion are above 94%, see figure 5. Compared with the blank group, the proportion of Bacteroidetes in the intestinal flora of the food product model mice was significantly reduced (P <0.01), from 72.6% to 32%, while the content of the LSQ group, JWXS group, and DP group were: 57.5%, 56.1%, and 40.7%; the proportion of Firmicutes increased significantly (P <0.01), from 18.4% to 48.8%, while the content of LSQ group, JWXS group, and DP group were: 23.2%, 28.5%, and 42.9%. All three administration groups alleviated this change to a certain extent.

At the genus level, the OTU annotations of the five groups are Bacteroides (8.39% to 22.23%), Lachnospiraceae NK4A136 group (4.12% to 15.48%), Prevotellaceae UCG-004 (3.10% to 5.80%), Helicobacter (0.67% to 5.83%), Lachnospiraceae UCG-008 (0.45% to 5.90%), Prevotellaceae UCG-001 (1.33% to 4.53%), Ruminococcaceae UCG-014 (0.56% to 2.40%), Anaerotruncus (0.38% to 4.25%), Lactobacillus (0.66% to 3.55%) and Parabacteroides (0.88% to 1.96%) mainly, see Figure 6,7. Compared with the blank group, the OTU in the model group was annotated as Lachnospiraceae NK4A136 group (P <0.01), Helicobacter (P <0.01), Lachnospiraceae UCG-008 (P<0.01), Anaerotruncus (P<0.01). Bacteroides decreased significantly (P<0.01), Prevotellaceae UCG-001 (P<0.01), and Lactobacillus (P <0.01) decreased significantly.

The correlation between 10 high-abundance phyla and the promotion of digestion and decreased gastric relaxation is shown in figure 8. It was found that in terms of promoting digestion, gastrin expression is highly correlated with Bacteroidetes. In terms of decreased gastric relaxation, the expression of cholinesterase has a high negative correlation with Verrucomicrobia.

Discussion

Loading [MathJax]/jax/output/CommonHTML/jax.js
Human gut microbes are the "second genome" of the human body \cite{18-20}. Changes in nutrient utilization and synthesis may accompany changes in the intestinal microbiota in species richness, diversity, composition, and function. These changes will have a profound impact on the host's physiological response \cite{21}. Many studies have shown that FD and digestive system dysfunction are closely related \cite{22}. MMF and many microorganisms, such as yeast and mold produced in the fermentation process, can improve the symptoms of intestinal microflora disorder in mice. It can regulate and protect the digestive system of animals and improve the intestinal flora \cite{23}.

Studies have shown that gut microbiota is closely related to indigestion \cite{24} FD patients will have substantial symptoms of fullness after a meal due to the delayed emptying of the stomach. At the same time, many findings using a gastric barostat have shown reduced proximal gastric relaxation in response to a meal in FD patients \cite{25,26}. Insufficient accommodation of the proximal stomach during and after the ingestion of a meal may be accompanied by increased intragastric pressure and activation of mechanoreceptors in the gastric wall, thus inducing symptoms \cite{27}. The pharmacological effect of MMF is mainly manifested in promoting food hydrolysis and improving gastrointestinal motility to enhance digestion \cite{28}. What's more, studies have shown that MMF can promote the movement of rat ileal smooth muscle \cite{29}. This study found that MMF, Jianweixiaoshi, and Domperidone can alleviate abdominal fullness symptoms in mice, and these changes may be related to the intestinal flora. Through the analysis of the relationship between the promotion of digestion, decreased gastric relaxation, and microbial community, it is found that MMF ability to improve intestinal ecology and promoting gastric emptying may have a specific correlation with intestinal flora and Bacteroidetes, but this needs to be confirmed by further experimental research.

Standard or "healthy" gut microbiota mainly include Firmicutes (about 50% to 75%) and Bacteroidetes (about 10% to 50%) \cite{18}. The Bacteroidetes of the dyspepsia mice was significantly reduced, which is consistent with the research results of Zhuang et al. \cite{30}, and MMF could reverse this change. The results of this study indicate that the increase in the diversity of intestinal microflora in mice with food accumulation may be related to disturbance of intestinal microbiota and increase of pathogenic bacteria (such as Helicobacter). Intestinal flora disorder inhibits the production and conversion of short-chain fatty acids (SCFA), one of the factors that induce FD. The most crucial SCFA in the intestine are acetic acid, propionic acid, and butyric acid \cite{31}. Short-chain fatty acids are also the main products of protein degradation and amino acid fermentation \cite{32}. Propionic acid-producing bacteria mainly belong to Bacteroidetes, and MMF can significantly increase the expression of Bacteroides. At the same time, this study found a negative correlation between Verrucomicrobia and cholinesterase. The muscle can relax afterward, rather than remain locked in a tense state; the acetylcholine must be broken down by a cholinesterase. The Verrucomicrobia of the dyspepsia mice is significantly increased, and MMF can reverse this change. MMF may restore the cholinesterase of dyspepsia mice by inhibiting the number of Verrucomicrobia, thereby obtaining proximal gastric relaxation and improving the symptoms of food accumulation.

Besides, studies have shown that MMF contains artemisinin, rutin, oleanolic acid, amygdalin, and quercetin, which have an excellent inhibitory effect on common intestinal pathogens, and it also has a good repair effect on the wounds on the surface of the digestive tract \cite{33,34}. It shows that traditional Chinese medicine MMF can increase the number of beneficial bacteria in the intestine, reduce the number of aerobic bacteria, and have a therapeutic effect on food accumulation.

In summary, MMF can play a therapeutic role by regulating the disturbance of intestinal flora in dyspepsia mice.
Conclusion

In this study, the 16S rRNA technique was used to provide an unexpected and unbiased analysis of the mechanism of action of MMF. MMF is a fermented medicine commonly used in Chinese medicine. First, assessing the changes in serum metabolites, we found that MMF has a specific effect on digestive function. Using high-throughput sequencing technology to detect intestinal microbes, we found that MMF plays a functional role in regulating intestinal microbes. It is consistent with traditional Chinese medicine theory, "MMF protects the spleen and stomach." By analyzing the correlation between 10 high-abundance bacteria and related factors of the digestive system, we further found that MMF plays a role in protecting the spleen and stomach and promoting digestion by regulating intestinal expression microorganisms such as Bacteroidetes and Verrucomicrobia.

The research provides a valuable reference model for explaining the theory of Chinese medicine, expands the potential applications of MMF, and provides reasonable goals and directions for further mechanism research.

Abbreviations

MMF: Massa MedicataFermentata
FD: Functional dyspepsia
FGID: Functional gastrointestinal disorder
SIBO: Small intestinal bacterial overgrowth
PCR: Polymerase chain reaction
TCM: Traditional Chinese Medicine
SPF KM: Specific-pathogen free Kunming
QIAGEN: QIAquick Gel Extraction Kit
PCoA: Principal coordinate analysis
SCFA: Short-chain fatty acids

Declarations

Funding

This study was supported by the Sichuan Provincial Administration of Traditional Chinese Medicine project - Key Technology Breakthrough and Quality Optimization Improvement of MMF (no.2018c022), Supporting Plan of Science and Technology Department of Sichuan Province (no.2016SZ0040) and Chengdu Science and Technology Huimin Project - Development and Research of Medicine and Food Homologous Traditional Chinese Medicine Health Tea (no. 2015-HM01-00401-SF).
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

XZ, HZ, QH, JW conceived and designed the study. XZ and HZ performed the experiments. XZ and HZ processed the data. XZ and HZ wrote the paper. All authors read and approved the manuscripts. All authors declare that they have no competing interests.

**Ethics approval and consent to participate**

All animals received humane care according to the Declaration of Helsinki, which was promulgated in 1964 and amended in 1996 (Moraru et al. 2014). All experimental protocols were approved by the Animal Care and Use Committee of Chengdu Municipal Hospital of Traditional Chinese Medicine, Chengdu, China (approval no. SCXK (Chuan) 2015-30).

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgments**

The experimental facilities were provided by the innovation Laboratory of the Chengdu University of Traditional Chinese Medicine. Thanks to Chengdu Rhonin Biosciences Co., Ltd. provides technical support.

**References**

1. Tack J, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR, Stanghellini V. Functional gastroduodenal disorders. Gastroenterology. 2006;130(5): 1466-1479.

2. Aro P, Talley NJ, Johansson SE, Agreus L, Ronkainen J. Anxiety is linked to new-onset dyspepsia in the Swedish population: a 10-year follow-up study. Gastroenterology. Gastroenterology. 2015;148(5): 928-937.

3. Wu BY, Zhang FC, Liang LX. Epidemiology of functional dyspepsia. Chin J Gastroenterol and Hepatol. 2013; 22(1): 85 -90.

4. Miwa H. Why dyspepsia can occur without organic disease: pathogenesis and management of functional dyspepsia. J Gastroenterol. 2012;47(8): 862-871.

5. Bures J1, Cyrany J, Kohoutova D, Forstl M, Rejchrt S, Kvetina J, Vorisek V, Kopacova M. Small intestinal bacterial overgrowth syndrome. World J Gastroenterol. 2010;16(24):2978-2990.
6. Dukowicz AC, Lacy BE, Levine GM. Small intestinal bacterial overgrowth: a comprehensive review. Gastroenterol Hepatol (N Y). 2007;3(2): 112422.

7. Saad RJ, Chey WD. Review article: current and emerging therapies for functional dyspepsia. Aliment PharmacolTher.2006;24(3): 475-492.

8. Ji XJ. Curative and preventive effects of Bifid Triple Viable Capsules combined with Mosapride on functional dyspepsia. Chin J Microecology. 2014; 26(5): 555-557.

9. Li Y, Xu XT, Zhang ZF, Li L, Yao QQ. Research progress on chemical constituents and biological activities of plants from HemsleyaCogn. Chin Tradit Herbal Drug. 2015;46: 2800-2809.

10. Moraru IG, Moraru AG, Andrei M, et al. Small intestinal bacterial overgrowth is associated to symptoms in irritable bowel syndrome. Evidence from a multicentre study in Romania. Rom J Intern Med. 2014; 52(3): 143 450.

11. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, and Knight R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 108 Suppl 1, 4516–22(2011).

12. Liu, C. et al. Long-term nitrogen addition affects the phylogenetic turnover of soil microbial community responding to moisture pulse. Scientific reports 7, 17492(2017).

13. Edgar, R. C. UPARSE: Highly accurate otu sequences from microbial amplicon reads. Nat Methods 10, 996–8(2013).

14. Edgar, R. C. Search and clustering orders of magnitude faster than blast. Bioinformatics 26, 2460(2010).

15. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2 - approximately maximum-likelihood trees for large alignments. PLOS ONE 5, e9490(2010).

16. R Core Team. R: A language and environment for statistical computing. (R Foundation for Statistical Computing, 2016).

17. Faith, D. Conservation evaluation and phylogenetic diversity. Biol Conserv61, 1–10(1992).

18. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. 2006. Metagenomic analysis of the human distal gut microbiome. Science. 2006;312(5778)1355-1359.

19. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307(5717)1915-1920.

20. Cani P D, Delzenne N M. Gut microflora as a target for energy and metabolic homeostasis. CurrOpin Clin NutrMetab Care.2007;10(6)729-934.

21. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013; 500(7464):541-546.

22. Fu DX, Chen HY. Clinical effect of bifidobacterium Lactobacillus triple live bacteria tablets combined with compound digestive enzyme capsules on children with functional dyspepsia. Henan Medical Research. 2020; 29(11): 2032-2033.

23. Zhu BY, Yang C, Jiang YY, Gou LQ, She QH, Zhang L. Research progress on digestive enzymes and biological activities of Liushenqu in traditional Chinese medicine. Molecular Plant Breeding. 2018; 16(11): 3763-3767.

24. Shi Y. Screening of Lactobacillus with the function of repairing antibiotics leading to intestinal dysbiosis and its mechanism. Jiangnan University, 2019.
25. Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology. 1998;115:1346–1352.

26. Salet GAM, Samsom M, Roelofs JMM, van Berge Henegouwen GP, Smout AJPM, Akkermans LMA. Responses to gastric distention in functional dyspepsia. Gut.1998;42:823–829.

27. Tack J, Bisschops R, and Sarnelli G. Pathophysiology and Treatment of Functional Dyspepsia. Gastroenterology. 2004; 127:1239–1255.

28. Gao PF, Zhang WY, Zhou RR, Zhang YC, Ma WW, and Shi XY. Effects of different Liushenqu on digestive function in mice, Chinese Archives of Traditional Chinese Medicine. 2016; 34(2): 362-364.

29. Shen XK, Zhang LR, Jiang GR, and Ye LL. Effect of different habitat Liushenqu on experimental animal intestinal, motion, Sichuan Medical Journal. 2010; 31(8):1061-1063.

30. Zhuang YH, Yang CH, Yang DX, Wang Y, Hu J, and Xia QP. Study on the microecological changes and curative effects of irritable bowel syndrome by Chinese drug "Shenqu" Chinese Journal of Microecology. 2005; 17(1): 41-43.

31. Sivaprakasam S, Prasad P D, Singh N. Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. PharmacolTher. 2016;164:144-151.

32. Chen Y, Cao YS, Liu XH. Short-chain fatty acids and intestinal flora. Jiangxi Science. 2006; 24(1): 38-49,69.

33. Qin XR, Zhang MJ, Gao XN, Lin Y, Ma L, and He SY. Study on the antibacterial activity of quercetin, Chemistry & Bioengineering. 2009; 26(4): 55-57, 78.

34. Guo LS, Yang XD, Hu J, Cai ZW, and Yang JY. The regulating and protecting effect of medicated leaven on intestinal flora imbalanced mice, Chinese Journal of Microecology. 2005; 17(3): 174-177.

Figures
Figure 1 Rarefaction curve of intestinal flora in the mouse from each group
Figure 2 Rank-Abundance curves of intestinal flora in the mouse from each group

Figure 2

Rank-Abundance curves of intestinal flora in the mouse from each group

Figure 3 PCoA score distribution of intestinal flora in the mouse from each group
Figure 3
PCoA score distribution of intestinal flora in the mouse from each group

Figure 4
Cluster Analysis Tree of intestinal flora in the mouse from each group
Figure 5 Relative abundance of community classification of intestinal flora in the mouse from each group at phylum level

Relative abundance of community classification of intestinal flora in the mouse from each group at the phylum level
Figure 6 Relative abundance of community classification of intestinal flora in the mouse from each group at genus level
Figure 7

Heatmap of species with high abundance of intestinal flora in the mouse from each group at the genus level.
Figure 8 Relationship between the promotion of digestion, decreased gastric relaxation and microbial community on intestinal flora in the mouse from each group at phylum level

Figure 8

Relationship between the promotion of digestion, decreased gastric relaxation and microbial community on intestinal flora in the mouse from each group at the phylum level.