MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic**Preliminary Isolation and Molecular Identification of Gut Microbiota in Mice with Enteritis**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-21
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APPROVE
Head of Biotechnology, Leather and Fur Department, Professor, Dr. Sc., Prof.
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«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Yang Yifan

1. Thesis topic **Preliminary Isolation and Molecular Identification of Gut Microbiota in Mice with Enteritis**

Scientific supervisor Dr. Sc., Prof. **Tetiana SHCHERBATIUK**

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2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

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WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	28 May 2025	
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I am familiar with the task:

Student _____ Yang Yifan

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Abstract

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Gastrointestinal flora imbalance is closely related to the occurrence and development of enteritis. However, the specific mechanism still requires in-depth study. In this research, a mouse model of enteritis induced by rhubarb was constructed. Selective media (EC, BPRMB, BS, LBS, EMB) were used to isolate the intestinal flora, and 16S rRNA gene sequencing technology was employed for molecular identification. The aim was to preliminarily analyze the structural characteristics of the intestinal flora under enteritis conditions. The experimental results showed that compared with the healthy control group, the diversity of the intestinal flora in enteritis mice was significantly reduced, the abundance of opportunistic pathogens (Escherichia coli) was significantly increased, while the proportion of potential probiotics (lactic acid bacteria) in the flora decreased. This study confirmed that the intestinal flora structure of enteritis mice was significantly different from that of the healthy state. Its separation and identification provided experimental basis and strain resources for subsequent exploration of the function of intestinal flora in enteritis and targeted intervention. The study also found that the intervention of Six God Flour could partially reverse the flora disorder and accelerate the proliferation of beneficial bacteria such as bifidobacteria.

Key words*: Enteritis; Intestinal flora; Isolation and culture; 16SrRNA gene sequencing; Mouse model*

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INTRODUCTION

This study aims to construct a mouse model of intestinal inflammation induced by rhubarb, isolate intestinal flora using selective culture media, and conduct molecular identification through DNA 16S gene sequencing technology. The objective is to preliminarily analyze the structural characteristics of intestinal flora under intestinal inflammation conditions, providing experimental evidence and bacterial resources for revealing the function of intestinal flora in intestinal inflammation and targeted intervention.

Chapter I

LITERATURE REVIEW

1.1 OVERVIEW AND PROGRESS OF RESEARCH ON INTESTINAL FLORA IN MICE

1.1.1 OVERVIEW

In view of the high similarity between itself and human intestinal flora, clear genetic background and convenient experimental operation, mice have become the core model animals in intestinal flora research. The high editability of mouse genome provides accurate means for analyzing the interaction mechanism between flora and host, such as verifying the function of specific strains by means of single bacteria colonization experiment [1]. Its short breeding cycle and intestinal physiological characteristics are very similar to human beings, which is convenient to simulate the pathological process of diseases[30] related to intestinal flora imbalance[29]. Standardized feeding conditions and controllable flora intervention measures can effectively eliminate the interference of environmental variables, ensure that the experiment can be repeated, and combine multi-omics technology with sterile mouse model to carry out work, researchers have revealed the intestinal flora of the host immune and metabolic regulation of the key pathways, further tamping the mouse in the intestinal microecology research irreplaceable position

1.1.2 RESEARCH STATUS

Intestinal flora plays a key role in maintaining the physiological balance of the host. Intestinal flora belongs to a very complex microbial community in animals. If the homeostasis of the flora is destroyed, it may induce the defects of the host's physiological development, immune abnormalities and abnormal nutritional metabolism[31], and also play a role in maintaining the intestinal barrier function, although through the combination of traditional culture methods and emerging omics techniques[32], researchers have reached a breakthrough understanding of the diversity of flora and its functions, but there are still obvious gaps in the existing knowledge system. It has been found that the imbalance of intestinal flora is closely related to the occurrence and development of many diseases, including obesity, diabetes, cardiovascular disease and intestinal related diseases. Enteritis is a 1 common intestinal disease[33], which greatly affects the health and quality of life of the host, and its pathogenesis is complex, In addition to factors such as heredity and environment, the change of intestinal flora plays a key role in the initiation, development and prognosis of enteritis. The incidence of ulcerative colitis (UC)[3] continues to rise in the world. The expression levels of PPARγ mRNA and protein in the colon of UC patients are significantly reduced. The expression of nitric oxide synthase 2(NOS2) and inducible nitric oxide synthase (iNOS) in the colonic epithelium of mice deficient in PPARγ is significantly increased[34], resulting in a significant increase in nitrate level in colonic mucosa[4]. This further causes the rapid abnormal proliferation of Enterobacteriaceae, blocks the growth of probiotics, and finally causes the imbalance of intestinal flora, which conveys that future research should focus on building a collaborative innovation model of culture omics and multi-omics technology[35], so as to promote the continuous expansion of microbial resource library and the deep breakthrough of functional decoding, further explore the intestinal flora of enteritis mice, and help to uncover the microbial mechanism of enteritis, it provides new ideas and targets for the prevention,

diagnosis and treatment of enteritis, which has important theoretical and practical significance.

Early studies on intestinal flora mainly rely on the traditional means of microbial culture, using the separation, culture and identification of intestinal microorganisms, began to know the composition and structure of intestinal flora, but the traditional culture method has some limitations, many intestinal microorganisms can not carry out in vitro culture, resulting in insufficient cognition of intestinal flora. With the rise of modern molecular biology techniques such as high-throughput sequencing technology, metagenomics and metabonomics, the study of intestinal flora has entered a new stage[5] it can carry out comprehensive and systematic research and analysis on intestinal flora, which reflects the diversity and complexity of intestinal flora; metagenomics can deeply analyze the gene function and metabolic pathway of intestinal flora; metabonomics can further grasp the interaction between intestinal flora and host by analyzing the metabolites of intestinal flora[36], The adoption of these technologies has greatly promoted the development of intestinal flora research, to make people's understanding of intestinal flora further in-depth and comprehensive.

1.2 RESEARCH PROGRESS ON THE RELATIONSHIP BETWEEN ENTERITIS AND INTESTINAL FLORA

Many studies have shown that the intestinal flora of enteritis patients or enteritis animal models has obvious imbalance. From the perspective of flora composition, the number of beneficial bacteria such as Bifidobacterium and Lactobacillus has decreased, while the number of harmful bacteria such as Escherichia coli and Enterobacter has been increasing[37]. The adjustment of the flora structure may destroy the balance of intestinal microecology and affect the normal physiological activities of the intestinal tract, thus causing the production and development of intestinal inflammation. Intestinal flora imbalance may also affect the intestinal immune function, metabolic function and

intestinal barrier function and other ways to participate in the pathological process of enteritis, intestinal flora produced by short chain fatty acids and other metabolites[6], Play the effect of regulating intestinal immunity and maintaining intestinal barrier function. If flora imbalance occurs, the production of short-chain fatty acids will decrease, which may cause intestinal immune function disorder and intestinal barrier function damage, leading to further aggravation of enteritis symptoms[38]. There is a complex interaction between intestinal flora and host immune system. Flora imbalance may cause abnormal immune response and further escalate intestinal inflammation.

1.3 CONSTRUCTION AND MANAGEMENT OF MOUSE MODEL OF ENTERITIS

1.3.1 TRADITIONAL CHINESE MEDICINE "RHUBARB" TO BUILD A MOUSE MODEL OF ENTERITIS

Rhubarb (Rheum palmatum L.) has long been famous for its purging, clearing away heat and detoxifying effects, and occupies a key position in the 1 law enforcement of traditional Chinese medicine[7]. Modern pharmacological research shows that there are anthraquinone compounds in rhubarb, and its purging effect is mainly caused by stimulating intestinal smooth muscle contraction and increasing secretion, thus inducing diarrhea and mucosal damage immediately[39], it is modelled on the partial pathological characterization of inflammatory bowel disease. In the study of network pharmacology combined with in vivo experiments, the researchers further verified by DSS model that the combination of rhubarb and Coptis chinensis can synergistically regulate inflammation-related pathways such as MAPK[40], indicating that it not only has laxative effect, but also can activate mucosal immune response. From the perspective of microecology, the administration of rhubarb can significantly change the structure of intestinal flora, mainly showing that the abundance of Bacteroides and Clostridium decreased, however, the number of Firmwall bacteria is increasing, resulting in a decrease in the output of short-chain fatty acids (SCFAs) [6], resulting in a weakening of

the nutritional support and barrier repair function of the intestinal mucosa[41], thereby exacerbating the process of inflammation. Rhubarb-induced pH changes coupled with mucus layer disruption can lead to downregulation of tight junction proteins (like ZO-1, Occludin) in intestinal epithelial cells[8], aggravating abnormal permeability and inflammatory cell infiltration, rhubarb presents a direct chemical purging effect, and with the help of multiple mechanisms such as slime, microecological imbalance and immune activation, a reproducible mouse model of enteritis with obvious pathological characteristics is jointly created, which provides a different perspective for the mechanism research of inflammatory bowel disease and the screening of new drugs.

1.3.2 "Six Divine Comedy" for the treatment of enteritis in mice

As a classic traditional Chinese medicine prescription of "eliminating food and guiding stagnation", Liushenqu is mainly composed of elements such as Shenqu, malt, radish seed, hawthorn, etc. This medicine can traditionally help digestion and relieve diarrhea in stomach[9]. Current research shows that fermented Liushenqu is rich in various digestive enzymes and beneficial microbial metabolites, which can improve intestinal digestion ability, promote flora balance, and help reduce intestinal disorder of food accumulation type. During the related animal experiments, Zhang Hongling and other members of the research group found that after feeding Liushenqu mice, the contents of gastrin and cholinesterase in serum increased significantly, and the content of nitric oxide (NO) in serum decreased[42], suggesting that Liushenqu not only promoted gastrointestinal motility, but also protected mucosa by inhibiting NO-mediated inflammatory pathway[10]. In the model of enteritis, the use of Liushenqu intervention can improve the disease activity index, reduce the degree of diarrhea and reduce the bleeding phenomenon that can be seen by the naked eye, which is closely related to its regulation of intestinal flora and help epithelial repair. A study with the help of metagenomic analysis showed that after the treatment of Liushenqu[43], the abundance of most bifidobacteria and lactobacillus increased, while the abundance of prexella and

streptococcus decreased significantly, telomerase activity and mucus secretion levels have been improved, confirming the existence of two-way regulation of intestinal microecology. Active ingredients such as hawthorn polyphenols and enzymatic hydrolysates of Divine Comedy can reduce the expression of pro-inflammatory factors such as TNF-α and IL-6[44], and can also up-regulate the expression of anti-inflammatory factors such as IL-10, and inhibit intestinal mucosal inflammatory response at the molecular level. As a representative prescription of "digestion and stagnation", Liushen Comedy not only has a reliable basis for improving gastrointestinal motility and improving symptoms of food accumulation, but also regulates anti-inflammatory flora, it provides a variety of theoretical reference for the treatment of mouse model of enteritis.

1.4 ISOLATION OF INTESTINAL FLORA

1.4.1 TRADITIONAL CULTURE SEPARATION

The traditional culture separation takes the microbial separation technology based on dilution and coating as the classical culture method. Its main operation is to inoculate the continuous dilution sample suspension on the surface of the solidified matrix[45] (generally using agar system. For special strains, different solid agents such as gellan gum or agarose can be selected[26]. Gellan gum relies on high transparency (light transmittance> 95%) and thermal stability (gel temperature 35 - 50 ℃), it has become the preferred carrier for strict visualization and separation of anaerobic bacteria, and agarose with low electroosmotic characteristics (electroosmotic value ≤ 0.13) is more suitable for subsequent molecular biological detection. At the level of target flora enrichment[46], researchers generally adopt multi-dimensional optimization methods: during nutrition supply, the adjustment of physical parameters involves the adjustment of temperature gradient (25 - 42 ℃), pH value (pH 5.0 - 8.5) and the control of gas atmosphere by means of simulating host microenvironment, at the same time, it is combined with selective inhibitors (such as 50 μg/mL vancomycin to inhibit gram-

positive bacteria) and growth cofactors (0.1mM heme, 5 μg/mL vitamin K3) to work together. Some research teams have innovatively integrated multi-omics technologies and successfully increased the pool of culturable species in human intestinal tract from 487 to 1057 by combining rapid identification of MALDI-TOF MS[11](sample mass spectrometry detection time is less than 10 minutes) and 16S rRNA gene barcode verification by establishing a standardized culture omics system containing 212 kinds of culture media, the newly discovered strains accounted for 61% of the total[28]. Despite a spate of new single-cell sorting techniques, the gradient dilution method[12]with the advantages of universal application of its equipment (only requiring ultra-clean table and incubator), cost control and customization of the scheme (supporting personalized additive combination), it is still the basic separation method for clinical microbiome research. However, due to in vitro culture conditions and the complexity of symbiotic bacteria interaction network, about 70% of intestinal microorganisms are still in a "non-culturable" situation.

1.4.2 MOLECULAR BIOLOGY SEPARATION

Metagenomics (Metagenomics), also known as microbial environmental genomics, directly extracts all microbial DNA from environmental samples and constructs a metagenomic library to analyze the species diversity and functional diversity of environmental samples in terms of species and gene function[14], molecular biology separation uses metagenome-assisted targeted separation strategy[13]. After targeting the target bacteria by high-throughput sequencing of 16S rRNA gene, specific labeling is implemented by coupling fluorescence activated cell sorting (FACS) technology, and living bacteria are separated by flow cytometry. Its separation efficiency is 3 - 5 times higher than that of traditional methods. It is worth noting that microfluidic chip technology developed in recent years[15] by constructing the intestinal bionic microenvironment, the dynamic capture and culture of strict anaerobic bacteria were

achieved at the single cell level, which provided a new template for breaking the technical bottleneck of intestinal flora separation.

1.5 IDENTIFICATION TECHNOLOGY OF INTESTINAL FLORA

1.5.1 WHOLE GENOME SEQUENCING

Whole Genome Sequencing (WGS) provides a highly sensitive analysis method for intestinal flora research by analyzing the complete gene content of microbial community. Unlike targeted sequencing technology, WGS can include the whole genome sequence of bacteria, archaea and fungi, while presenting the diversity of functional genes (such as metabolic pathways, antibiotic resistance genes) and non-coding regulatory elements. Using the strategy of shotgun sequencing[25] and the cooperation of Illumina NovaSeq or Oxford Nanopore platform, researchers can reconstruct almost complete microbial genome sketches from metagenome samples. Relying on the binning algorithm of deep learning, the identification efficiency of low abundance strains in complex samples is obviously improved. WGS is hindered by its high demand for computing resources and high sequencing cost, which hinders its popularization in large cohort studies, in the future, we can rely on high technology and algorithm optimization to further reduce the threshold of analysis.

1.5.2 16S rRNA GENE AMPLICON SEQUENCING

The precise identification technology system of intestinal flora has been advanced from the traditional phenotype analysis to the intelligent identification system combined with multi-omics. The traditional identification mostly relies on the morphological observation of selective medium combined with biochemical reaction detection, but its discrimination ability can only reach the genus level. The 16SrRNA gene amplicon sequencing is based on the highly conservative characteristic of bacterial ribosomal

RNA gene [20], and the variable region (such as V3-V4 or V4-V5) is amplified by PCR, in this way, high-throughput detection of flora composition is achieved [16]. However, its classification resolution is generally limited to the level of bacterial genera and above, and it is difficult to achieve accurate discrimination at the species level or subspecies level. This technology uses platforms such as Illumina MiSeq to sequence amplified products, and analyzes the differences in microbial diversity between samples by clustering the classification units (OTUs) [18] or amplified sequence variants (ASVs) [17]. This technique shows significant advantages in the preliminary screening of pre-culture samples, especially the rapid analysis of complex microbial communities. Compared with whole genome sequencing, its cost-effectiveness performance is better. It can be applied to large-scale clinical cohort or ecological studies. Primer selection bias may make the amplification efficiency of specific flora worse, and the resolution is mostly limited to the genus level. This gene sequence has 10 conserved domains and 9 hypervariable regions [47], and the conserved regions can provide anchor sites for primer design. The differences in nucleotides in the hypervariable regions are molecular markers for species identification. Universal primers are designed with reference to the conserved regions for targeted amplification. After high-throughput sequencing is implemented, the sequence data are matched with standard databases such as Greengenes and SILVA by using bioinformatics analysis procedures [19] in order to determine the classification of microorganisms, the species annotation approach based on machine learning (such as the q2-feature-classifier plug-in in QIIME 2[48]) significantly improves the accuracy of classification, but it still has to be combined with functional prediction tools to indirectly calculate metabolic potential.

1.5.3 MALDI-TOF MASS SPECTROMETRY

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) achieves the rapid identification of intestinal flora by detecting the specific protein fingerprint of microorganisms [21]. Compared with traditional biochemical identification methods, this technology can complete the identification of

strains in a few minutes without setting up pre-culture links, which is a rapid identification technology relying on the characteristics of proteome, MALDI-TOF MS overcomes the time bottleneck of traditional phenotypic analysis (such as biochemical reaction identification) [22] and can reduce the microbial identification process from days to minutes, while significantly reducing costs. Its core principle is to realize accurate identification of strain level by detecting characteristic polypeptide spectrum of microorganisms[49]. The technology can realize sub-microgram detection sensitivity, generate specific fingerprint with only a small amount of pure culture, realize peak map feature matching by constructing standardized database (such as Bruker MBT Smart)[50], and MALDI-TOF MS has an accuracy rate of over 90% in identifying common intestinal bacteria. However, its limitation is that it has limited ability to identify rare bacteria or unincluded strains, and it is difficult to identify highly homologous related species[23] Researchers analyzed five different types of diarrheagenic Escherichia coli (EAEC, ETEC, EIEC, EHEC, EPEC) with the help of MALDI-TOF technology, and found specific biomarker peaks[26]. The latest research has gradually improved the sensitivity of low abundance bacteria detection in complex samples with the help of liquid chromatography (LC-MALDI) and artificial intelligence-assisted peak map analysis[51], and may promote its application in precision medicine. With the continuous expansion of intestinal microbial reference database, this technology has the potential to become the core identification platform for large-scale microbial isolation and culture experiments.

1.6 RESEARCH PURPOSE AND SIGNIFICANCE

The goal of this study is to carry out preliminary isolation and molecular identification of intestinal flora in mice with enteritis, analyze the composition and structural characteristics of intestinal flora in **mice** with enteritis, and study the relationship between intestinal flora and the onset and development of enteritis, for example, 16SrRNA gene sequencing and other means to carry out molecular identification of isolated flora[24] finally, the results of isolation and identification were

analyzed to explore the change trend of intestinal flora in mice with enteritis and its association with enteritis.

Summary of the chapter I

This study focused on the intestinal microbiota of mice with enteritis, using mice as the core model organism (as they have a high similarity to the human intestinal microbiota, clear genetic background, and are easy to handle). The enteritis model was constructed by inducing with rhubarb, and the microbiota was isolated using selective media such as EC and BPRMB. Molecular identification was carried out using techniques such as 16S rRNA gene sequencing. The study found that the diversity of the microbiota in mice with enteritis decreased, the abundance of pathogenic bacteria such as Escherichia coli increased, and the proportion of probiotic bacteria such as Lactobacillus decreased. The intervention of Six God Flax could partially reverse the microbiota disorder. At the same time, the microbiota was isolated using traditional culture and molecular biology methods, and identified using techniques such as whole genome sequencing, 16S rRNA gene amplification sequencing, and MALDI-TOF mass spectrometry. The aim was to analyze the structural characteristics of the intestinal microbiota under enteritis conditions, and to provide experimental basis and bacterial strain resources for revealing the function of the microbiota in enteritis and targeted intervention

Chapter II

OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 EXPERIMENTAL MATERIALS AND INSTRUMENTS

2.1.1 MAIN MATERIALS

several mice

2.1.2 EXPERIMENTAL REAGENTS AND INSTRUMENTS

PCR instrument (ABI), electrophoresis instrument (Beijing 61 Instrument Factory), centrifuge (Eppendorf), agarose (Sun Horse), water bath pot (Shanghai Jinghong Experimental Equipment Co., Ltd.), clean bench (Shanghai Jinghong Experimental Equipment Co., Ltd.), biochemical incubator (Beijing 61 Instrument), 9cm-10cm diameter glass petri dish, applicator, pipette, bacterial genomic DNA extraction kit

2.2 EXPERIMENTAL STEPS

2.2.1 CONSTRUCTION OF ANIMAL MODEL BY FEEDING CHINESE MEDICINE "RHUBARB" FEED

Establishment of a mouse model of enteritis by feeding mice with traditional Chinese medicine "rhubarb"

2.2.2 PREPARATION OF CULTURE MEDIUM FOR ISOLATION AND IDENTIFICATION OF MOUSE INTESTINAL FLORA

(1) The medium required for the experiment is as follows:

EC Broth Medium:

Purpose: Used for the determination of fecal coliform and Escherichia coli.

Usage: Weigh 37.0g of this product, heat, stir, dissolve in 1000ml distilled water, sub-pack into 16mm * 150mm test tubes with inverted fermentation tubes, 8ml per tube, and autoclaved at 121 ℃for 15 minutes for later use.

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-1EC Broth Medium Composition and Content

EC Broth Medium	Content g/L
Tryptone	20.0
Lactose	5.0
Sodium chloride	5.0
dipotassium hydrogen phosphate	4.0
potassium dihydrogen phosphate	1.5
No. 3 bile salt	1.5
pH	6.9±0.1

Bacteroides fragilis activation medium broth

Product use: For Bacteroides fragilis activation culture.

Product usage: weigh 29.4g of this product, heat and dissolve it in 1000ml distilled water, sub-package, autoclaved at 121 ℃ for 15min, and set aside.

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-1Bacteroides fragilis activation medium broth BPRMB medium composition and content

Bacteroides fragilis activation medium broth BPRMB liquid	Content g/L
meat infusion	10
cheese peptone	10
Yeast Cream	2
Sodium chloride	5.0
L-Cysteine monohydrate	0.5
Glucose	1.8
Magnesium sulfate heptahydrate	0.12
calcium chloride dihydrate	0.05

Bifidobacterium BS medium

Product use: used for bifidobacterium isolation and culture.

Product usage: weigh 68.9g of this product, absorb Tween 80 1ml, heat, stir and dissolve in 1000ml distilled water, autoclave at 116 ℃ for 30 minutes, cool to 50-55 ℃, pour into a sterile plate.

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-3Culture medium composition and content of Bifidobacterium BS

Bifidobacterium BS medium	Content g/L
Peptone	10
liver immersion powder	5
beef dipped powder	3
yeast dip powder	5.0
Tryptone	8
soluble starch	0.5
Sodium chloride	1
dipotassium hydrogen phosphate	1
potassium dihydrogen phosphate	1
Glucose	10
Ferrous sulfate heptahydrate	0.01
manganese sulfate	0.005
L-Cysteine	0.5

Bifidobacterium BS medium	Content g/L
Agar	20
pH	7.2±0.1

LBS agar

Product use: for lactic acid bacteria detection and isolation culture

Product usage: weigh 84.0g of this product, then absorb Tween 80 1ml and glacial acetic acid 1.3ml, heat and stir and dissolve in 1000ml distilled water. use it on the same day without autoclaving. Use the next day, need 118 ℃ autoclaving 15 minutes.

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-4LBS agar medium composition and content

LBS agar	Content g/L
yeast dip powder	5
Tryptone	10
potassium dihydrogen phosphate	6
Ferrous sulfate	0.034
Magnesium sulfate	0.575
Glucose	20
Sodium acetate	25
Ammonium citrate	2
manganese sulfate	0.12
Agar	15
pH	5.5±0.2

Eosin methylene blue agar (EMB)

Product use: Weak selective medium for the separation of intestinal pathogenic bacteria, especially E. coli.

Product usage: weigh 37.4g of this product, dissolve it in 1000ml distilled water by heating and stirring, pack it into triangular bottles, and sterilize it under high pressure at 121 ℃ for 15 minutes for later use.

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-5Composition and content of eosin methylene blue agar medium

Eosin methylene blue agar (EMB)	Content g/L
Peptone	10
Lactose	10
dipotassium hydrogen phosphate	2
Agar	15
Ehong	0.4
Meilan	0.065
pH	7.0±0.2

(2) Configuration of culture medium

1. preparation of 100ml medium

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-6100ml Medium Composition and Content of

Medium	g/L	Preparation of 100mL	
EC Liquids	37.0	3.7	
BPRMB LIQUID	29.4	2.94	
BS solid	68.9	6.89	
LBS solid	84	8.4	Tween 80,1mL/L, glacial acetic acid 1.3ml/L
EMB solid	37.4	3.74	

2.According to the formula, after regular sterilization plate

Table **Ошибка! Текст указанного стиля в документе отсутствует.**-7Number of Configured Media

100ml medium	9cm Petri dish 25ml spread/piece
EC Liquids	20

100ml medium	9cm Petri dish 25ml spread/piece
BPRMB LIQUID	20
BS solid	20
LBS solid	20
EMB solid	20

3. Place the plate at 37 ℃, incubate at constant temperature for 12-48h, detect colony formation and turbidity in liquid culture medium, and visually inspect whether the thallus grows.

Figure**Ошибка! Текст указанного стиля в документе отсутствует.**. 1configured media

2.2.3 ISOLATION AND CULTURE OF INTESTINAL MICROBES

After the experimental mice were intervened by drugs (rhubarb, etc.) for 10 consecutive days, anatomical sampling was carried out on the 11th day. The experimental personnel wear sterile gloves in the biological safety cabinet. The specific operation is as follows:

(1) Immediately after removing the mouse colon intact, the colon length (accurate to 0.1mm) was measured and recorded using a precision vernier caliper. Then, the colon is longitudinally dissected along the mesentery, and the pre-cooled sterile PBS buffer is used for three pulsed washings, each with a volume of 5mL for 30 seconds. After the surface moisture is removed by sterile filter paper, the colon is placed in a pre-weighed

sterile petri dish, and 50-100mg of tissue sample is accurately weighed with a precision electronic balance.

(2) Treatment of intestinal contents flora: Take intestinal contents, add 0.5mL glycerol and store at 20 ℃. According to 1mL/1g intestinal contents, add PBS, fully homogenate and resuspend: centrifuge at 4000-5000rpm for 10s; Take 200ul of upper liquid as mother liquor; Dilute 10 times according to 200ul mother liquor + 1800ul PBS to 10-6 concentration gradient; Select 10-3,10-4,10-5,10-6 four concentration gradients, mark 3/4/5/6 in the number, and coat the bacterial solution 150ul/plate; After 1h, put it into a 37 ℃ biochemical incubator for constant temperature culture, and take photos to record the colony formation after 12h.

(3) Coat flat plates, and do a parallel repeat for each flat plate, as shown in the following table:

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-2coated five media plates-control and rhubarb-treated mice gut microbiota

Name of experimental group	Medium	Name							
CK	BP6.5	CK3A	CK4A	CK5A	CK6A	CK3B	CK4B	CK5B	CK6B
	BS6.5	CK3A	CK4A	CK5A	CK6A	CK3B	CK4B	CK5B	CK6B
	EC6.5	CK3A	CK4A	CK5A	CK6A	CK3B	CK4B	CK5B	CK6B
	EMB6.5	CK3A	CK4A	CK5A	CK6A	CK3B	CK4B	CK5B	CK6B

Name of experimental group	Medium	Name							
	LBS6.5	CK3 A	CK4 A	CK5 A	CK6 A	CK3 B	CK4 B	CK5 B	CK6 B
DH	BP6.5	DH3 A	DH4 A	DH5 A	DH6 A	DH3 B	DH4 B	DH5 B	DH6 B
	BS6.5	DH3 A	DH4 A	DH5 A	DH6 A	DH3 B	DH4 B	DH5 B	DH6 B
	EC6.5	DH3 A	DH4 A	DH5 A	DH6 A	DH3 B	DH4 B	DH5 B	DH6 B
	EMB6.5	DH3 A	DH4 A	DH5 A	DH6 A	DH3 B	DH4 B	DH5 B	DH6 B
	LBS6.5	DH3 A	DH4 A	DH5 A	DH6 A	DH3 B	DH4 B	DH5 B	DH6 B

Table Description: Capital letters of culture medium abbreviation; 6.5 means June 5, 2013; CK means blank control, normal saline group; DH means rhubarb treatment group without drug treatment group; A and B are parallel repeat group.

2.2.4 ISOLATION, CULTURE AND IDENTIFICATION OF INTESTINAL FLORA IN DRUG-TREATED MICE (LIUSHENQU DRUG TREATMENT)

The following table statistics are made for the delivered samples

Select typical values and take individual samples for testing, totaling 7 groups.

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-3 Sample Number Statistics and Weights

Sample No.	Weight of contents/g		Sample No.	Weight of contents/g
2.1	0.2		6.1	0.3
2.2	0.29		6.2	0.22
2.3	0.1		6.3	0.3
			6.4	0.31
3.1	0.43		6.5	0.15
3.2	0.76			
3.3	0.8		7.1	0.26
3.4	0.5		7.2	0.15
3.5	0.4		7.3	0.287
3.6	0.34		7.4	0.134
3.7	0.68		7.5	0.136
4.1	0.685		8.1	0.17
4.2	0.3		8.2	0.38
4.3	0.331		8.3	0.46
4.4	0.052		8.4	0.35
4.5	0.101			
4.6	0.328			
5.1	0.4			
5.2	0.48			
5.3	0.29			
5.4	0.45			
5.5	0.56			

Take the required sample for dilution

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-4Sample Dilution

Sample No.	Weight of contents/g	Add PBS/ul	/ul after centrifugation	Add PBS to dilute 20 times/ul
2.1	0.2	400	50	950

Sample No.	Weight of contents/g	Add PBS/ul	/ul after centrifugation	Add PBS to dilute 20 times/ul
3.1	0.43	860	50	950
4.3	0.331	662	50	950
5.1	0.4	800	50	950
6.1	0.3	600	50	950
7.1	0.26	520	50	950
8.4	0.35	700	50	950

After dilution, divided into two groups, A group to take 25ul coating plate; B group to take 50ul coating.

medium preparation steps:

Prepare culture medium, 400ml culture medium, prepare and place at 121 ℃, sterilize for 20min.

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-5Configuration 400ml Medium Composition and Content

Medium	g/L	Prepare 400mL, take/g	Add
EC Liquids → Solids	37.0	14.8	4g agar powder
BPRMB Liquid → Solid	29.4	11.8	4g agar powder
BS solid	68.9	27.6	
LBS solid	84	33.6	Tween 80,1mL/L,400ul Glacial acetic acid 1.3ml/L,420ul
EMB solid	37.4	15.0	

2.80 sets of glass petri dishes for regular and humid heat sterilization, 70 sets of actual use and 10 sets of spare.

3. When the culture medium is cooled to a little hot, pour the plate, 20mL/plate, and prepare 14 plates for each culture medium, totaling 14 × 5=70 plates.

(3) Sample dilution experiment steps:

1. Take samples from the corresponding marks of the freezing chamber at the bottom of Haier refrigerator: 2.1, 3.1, 4.3, 5.1, 6.1, 7.1, 8.4 (actual marks 2-1...);

2. Place it at room temperature for 15min, and add PBS to the above table in the sample without tube for dilution;

3. Mix well, centrifuge at 4000rpm for 30s, and add 50ul of supernatant into new tubes respectively;

4. Add 450ul PBS to each tube to dilute, and finally obtain 500ul/tube;

5. Mark the plate as shown in the following table;

6. Group A takes 25ul coated plate; Group B takes 50ul coated plate;

7. Dry after coating, and culture in a 37 ℃ incubator for 48h;

2.2.5 BACTERIAL GENOMIC DNA EXTRACTION AND IDENTIFICATION

First, the whole genome of the pure culture strain was extracted by bacterial genomic DNA extraction kit, and the quality of the extracted DNA was identified by agarose gel electrophoresis. Then the existing amplification primers were used for PCR amplification, and then the PCR products were electrophoresed, and the samples with clear target bands were selected for purification. The positive samples of PCR products were sequenced, and the sequencing results were identified by bacterial classification after sequence alignment. This part of the experiment was entrusted to a sequencing company to analyze the successful sequencing results of the feedback.

Summary of chapter II

1. The animal model was constructed by feeding with the Chinese medicine "Rhubarb" feed.

2. The intestinal microbiota of mice was isolated, cultured and identified. The culture medium for EC broth, activated culture medium broth for Bacteroides fragilis, Bifidobacterium BS medium, LBS agar, eosin-methylene blue agar, etc. were prepared according to the formula and sterilized.

3. After intervention with rhubarb on mice, the intestines were dissected and sampled. The intestinal contents were processed, the bacterial liquid was diluted and spread on the culture medium plates. After cultivation, the formation of colonies was observed.

4. The intestinal microbiota of mice was isolated and cultured and identified after drug treatment (treatment with Six God Flour drug). The samples were diluted, the culture medium was prepared, the plates were coated and cultivated.

5. The DNA of the pure culture strains was extracted, and PCR amplification, electrophoresis, purification and sequencing were performed. The sequences were compared for bacterial classification and identification.

Chapter III

EXPERIMENTAL PART

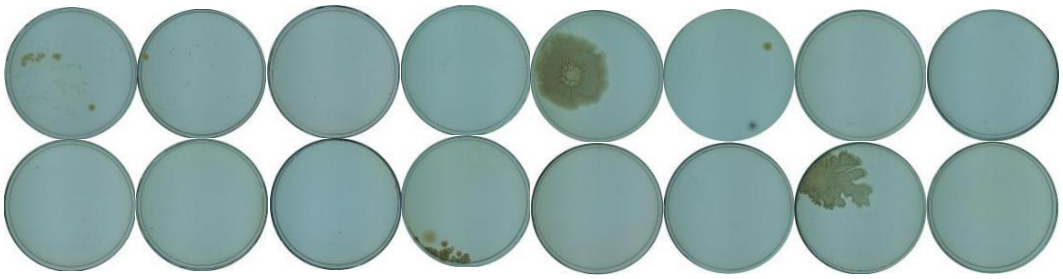
3.1 ISOLATION AND PURIFICATION OF BACTERIA

EC, BPRMB, BS, LBS and EMB5 kinds of medium for aerobic isolation and culture of intestinal flora. After 5 rounds of purification to pick a single colony of different shape, color and size.

3.2 EXPERIMENTAL RESULTS OF MEDIUM CONTROL GROUP

12h-16h,37 ℃ culture plate did not appear any colonies, proving that the culture medium and culture conditions are pollution-free, can carry out the next colony culture experiment.

3.3 EXPERIMENTAL RESULTS OF INTESTINAL FLORA IN CONTROL GROUP AND RHUBARB-TREATED MICE



Figure**Ошибка! Текст указанного стиля в документе отсутствует.**.1 BP6.5 (left 8 culture media are CK group, right 8 culture media are DH group)

Figure**Ошибка! Текст указанного стиля в документе отсутствует.**. 2BS6.5 (left 8 culture media are CK group, right 8 culture media are DH group)

Figure**Ошибка! Текст указанного стиля в документе отсутствует.**. 3EC6.5 (left 8 media for CK group, right 8 media for DH group)

Figure **Ошибка! Текст указанного стиля в документе отсутствует.**EMB6.5. 4(left 8 culture medium for CK group, right 8 culture medium for DH group)

Figure**Ошибка! Текст указанного стиля в документе отсутствует.**. 5LBS6.5 (left 8 culture medium for CK group, right 8 culture medium for DH group)

3.4 ISOLATION, CULTURE AND IDENTIFICATION OF INTESTINAL FLORA IN MICE (DRUG TREATMENT OF LIUSHENQU)

Table**Ошибка! Текст указанного стиля в документе отсутствует.**-6of Liushenqu drug treatment of enteritis in mice intestinal flora culture medium flora identification

B Parallel repeat experimental group	Medium	Name and Results						
A	BP	2.1A	3.1A positive miscellaneous Bacteria/Fungi	4.3A Less viable bacteria air bubble	5.1A	6.1A	7.1A positive bacteria or fungi	8.4A
	BS	2.1A Bud Sporium	3.1A bacillus	4.3A Not clear	5.1A Unknown	6.1A	7.1A bacillus Mold	8.4A
	EC	2.1A	3.1A	4.3A	5.1A	6.1A air bubble	7.1A	8.4A

B Parallel repeat experimental group	Medium	Name and Results						
						three kinds of bacteria		
	EMB	2.1A	3.1A The hollow is Bubble	4.3A	5.1A	6.1A	7.1A	8.4A air bubble three kinds of bacteria
	LBS	2.1A	3.1A	4.3A	5.1A	6.1A	7.1A	8.4A
B	BP	2.1B	3.1B a variety of bacteria	4.3B positive bacteria	5.1B bacillus	6.1B	7.1B bacillus	8.4B
	BS	2.1B	3.1B bacillus	4.3B	5.1B	6.1B	7.1B	8.4B
	EC	2.1B	3.1B	4.3B	5.1B	6.1B	7.1B	8.4B Countless small colony

B Parallel repeat experimental group	Medium	Name and Results						
	EMB	2.1B	3.1B Hollow is Bubble	4.3B air bubble Small bacteria	5.1B	6.1B	7.1B	8.4B Fungi two kinds of bacteria
	LBS	2.1B	3.1B	4.3B	5.1B	6.1B	7.1B	8.4B positive bacteria or fungi

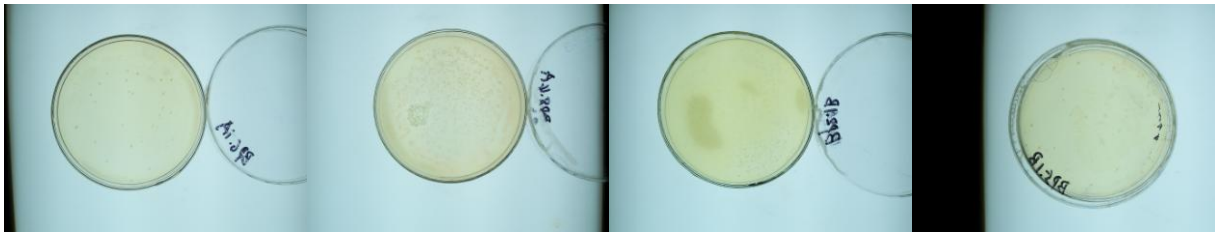


Figure **Ошибка! Текст указанного стиля в документе отсутствует.**.6 BP group culture medium.

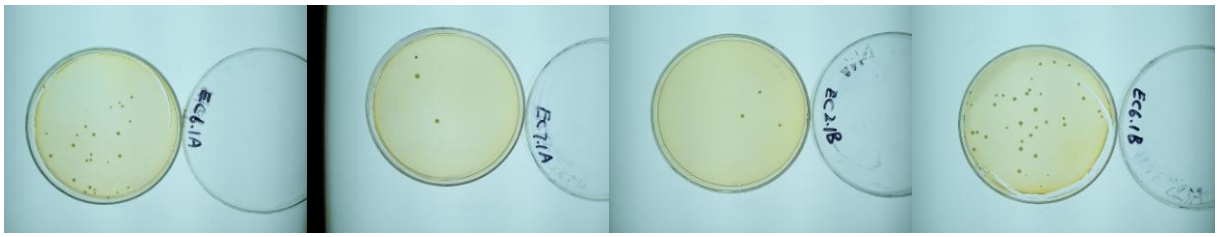
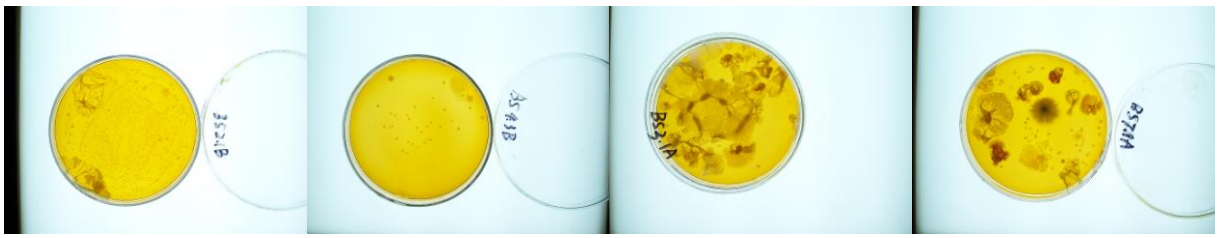


Figure **Ошибка! Текст указанного стиля в документе отсутствует.**.7 EC medium.



Figure**Ошибка! Текст указанного стиля в документе отсутствует.**. 6BS medium

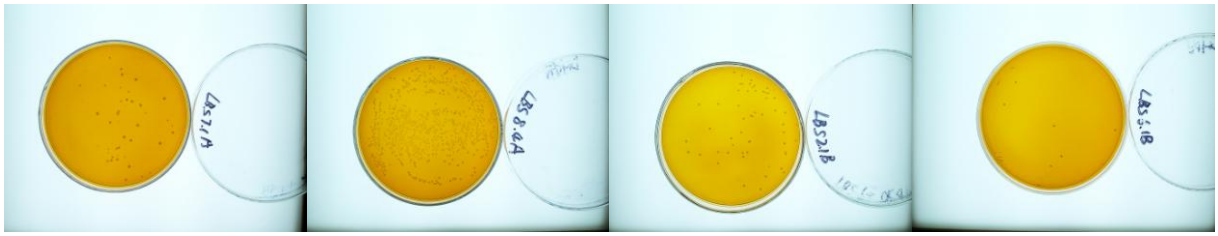


Figure **Ошибка! Текст указанного стиля в документе отсутствует.**.9 LBS medium.

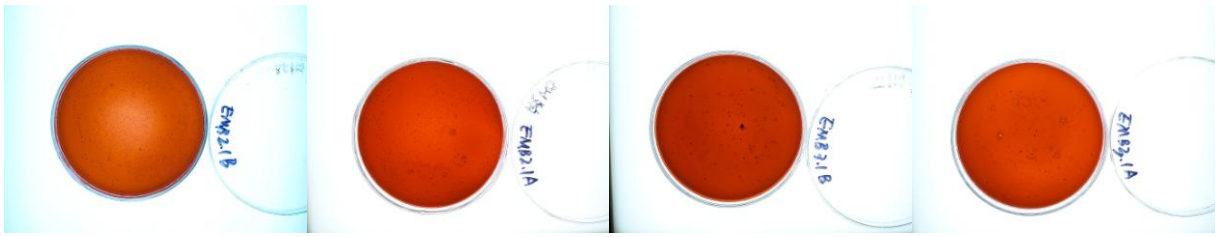


Figure **Ошибка! Текст указанного стиля в документе отсутствует.**.10 EMB medium.

3.5 BACTERIAL DNA 16S SEQUENCING RESULTS

Ribosomal DNA 16S sequencing was performed on one of the isolated bacteria. The sequence is as follows:

TGGGGGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGAT
GGTGCTTGCACCTGATTGACGATGGATTACCAGTGAGTGGCGGACGGGTGAG
TAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATG

CTAATACCGCATAACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTT
GGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACG
GCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAAT
GGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCACTAGGGAATCTTC
CACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTT
TCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTT
CACGCAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCA
GCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGC
GAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAG
AAGTGCATCGGAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTC
CATGTGTAGCGGTGGAATGCGTAATATATGGAAGAACACCAGTGGCGAAGGC
GGCTGTCTGGTCTGCAACTGACACTGAGGCTCGAAAGCATGGGTAGCGAAC
AGGATTAGATACCCTGGTAGTCCATGCCGTATACGATGAGTGCTAGTGTTGGA
GGGTTTCCGCCCTTCACTGCCGGAGCTAACGCATTAAGCACTCCACCCTGGG
GAGTACGACCGCAAGGTTGAAACTCGAAGGAATTGACGGGGGCCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCCAAAGCTACTCGAAAAACCTTACCAGG
ACTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTCCCTTCGAGGACCCAAT
GACAGGTGGTGCATGGTTCCTCGTCAGCT

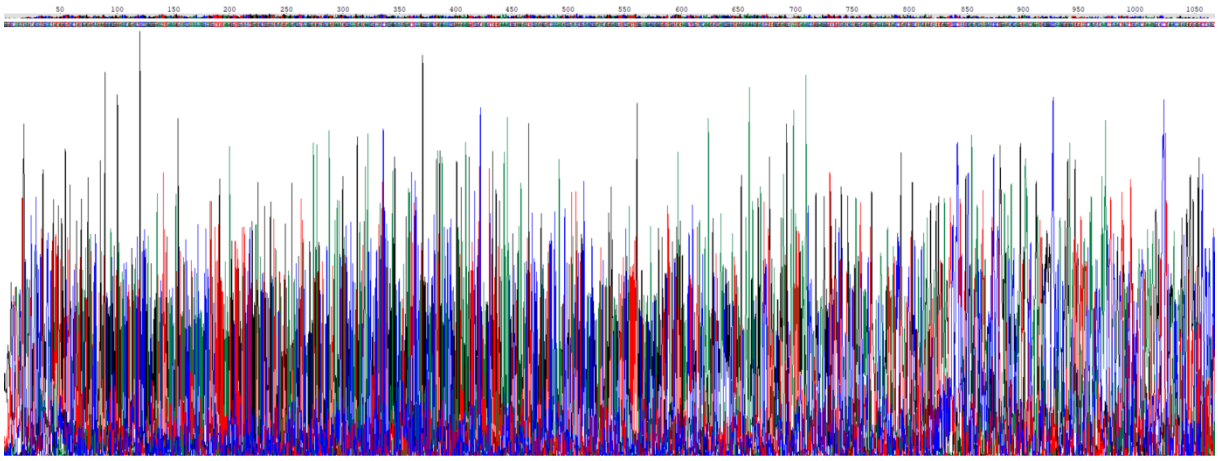


Figure **Ошибка! Текст указанного стиля в документе отсутствует.**.11 peak of bacterial ribosome DNA 16S sequencing sequencing

Sequences producing significant alignments Download Select columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewe

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Limosilactobacillus reuteri strain 2010 Contig_30, whole genome shotgun sequence	Limosilactobac…	1633	1633	86%	0.0	98.80%	162381	NZ_PUXG01000006.1
Limosilactobacillus reuteri strain 2010 Contig_14, whole genome shotgun sequence	Limosilactobac…	1700	1700	90%	0.0	98.55%	12155	NZ_PUXG01000022.1
Limosilactobacillus reuteri strain 2010 Contig_38, whole genome shotgun sequence	Limosilactobac…	1838	1838	100%	0.0	97.93%	275247	NZ_PUXG01000035.1
Limosilactobacillus agrestis strain WF-MT5-A scaffold71, whole genome shotgun sequence	Limosilactobac…	1838	1838	100%	0.0	97.85%	61844	NZ_JACIVF010000071.1
Limosilactobacillus balticus strain BG-AF3-A scaffold23, whole genome shotgun sequence	Limosilactobac…	1461	1461	80%	0.0	97.66%	1355	NZ_JACIVH010000023.1
Limosilactobacillus agrestimuris strain DSM 106037 NODE_24_length_1331_cov_381.722488, wh…	Limosilactobac…	1363	1363	76%	0.0	97.16%	1331	NZ_CALPCW010000024.1
Limosilactobacillus albertensis strain Lr3000 scaffold75, whole genome shotgun sequence	Limosilactobac…	1766	1766	100%	0.0	96.63%	50110	NZ_JACIVD010000075.1
Limosilactobacillus rudii strain STM3_1 scaffold3, whole genome shotgun sequence	Limosilactobac…	1507	1507	86%	0.0	96.51%	972	NZ_JACIVB010000003.1
Limosilactobacillus urinaemulieris isolate Limosilactobacillus_urinaemulieris_DSM106190, whole g…	Limosilactobac…	1456	1456	83%	0.0	96.50%	1419	NZ_CAKOBX010000019.1
Limosilactobacillus walteri strain M31 LR31_S1_L001_R1_001__paired__contig_62, whole genom…	Limosilactobac…	1744	1744	100%	0.0	96.34%	1780	NZ_QORN01000062.1
Limosilactobacillus viscerum strain CIEM62 NODE_68_length_971_cov_3060.243848, whole gen…	Limosilactobac…	1426	1426	82%	0.0	96.23%	971	NZ_CANDNW010000068.1
Limosilactobacillus caccae strain Marseille-P3519T, whole genome shotgun sequence	Limosilactobac…	1733	1733	100%	0.0	96.07%	909365	NZ_FTOY01000011.1
Limosilactobacillus frumenti strain LF145 chromosome, complete genome	Limosilactobac…	1683	6711	100%	0.0	95.31%	1752928	NZ_CP044534.1
Limosilactobacillus portuensis strain LV1276_C155 chromosome, complete genome	Limosilactobac…	1683	8411	100%	0.0	95.23%	1928671	NZ_CP117296.1
Limosilactobacillus vaginalis strain AM_LB9 chromosome, complete genome	Limosilactobac…	1677	8361	100%	0.0	95.14%	1880369	NZ_CP104399.1
Limosilactobacillus pontis strain LP475 chromosome, complete genome	Limosilactobac…	1674	10015	100%	0.0	95.14%	1714771	NZ_CP045530.1
Limosilactobacillus avistercoris strain Sa3CUN2 NODE_48_length_1780_cov_958.480916, whole …	Limosilactobac…	1672	1672	100%	0.0	95.13%	1780	NZ_JACSQW010000048.1
Limosilactobacillus galli strain BC6G17 NODE_94_length_1476_cov_1288.130093, whole genom…	Limosilactobac…	1666	1666	100%	0.0	95.04%	1476	NZ_CANCWK010000094.1
Limosilactobacillus fastidiosus strain WF-MO7-1 scaffold16, whole genome shotgun sequence	Limosilactobac…	1661	1661	100%	0.0	94.94%	1701	NZ_JACIUZ010000016.1
Limosilactobacillus kribbianus strain YH-lim2214 contig59, whole genome shotgun sequence	Limosilactobac…	1530	1530	93%	0.0	94.57%	10453	NZ_JAOVYZ010000059.1
Limosilactobacillus oris strain AM_LB10 chromosome, complete genome	Limosilactobac…	1616	8073	100%	0.0	94.11%	2007883	NZ_CP104398.1
Limosilactobacillus antri DSM 16041 SCAFFOLD1, whole genome shotgun sequence	Limosilactobac…	1616	1616	100%	0.0	94.11%	754183	NZ_GG700732.1
[Lactobacillus] timonensis strain Marseille-P3825, whole genome shotgun sequence	[Lactobacillus] …	1572	4712	100%	0.0	93.45%	1623765	NZ_LT964761.1
Limosilactobacillus secaliphilus strain DSM 17896 NODE_2, whole genome shotgun sequence	Limosilactobac…	1572	1572	100%	0.0	93.38%	1631	NZ_JQBW01000001.1
Limosilactobacillus caecicola strain DSM 110982 NODE_8_length_1715_cov_1695.676435, whole…	Limosilactobac…	1561	1561	100%	0.0	93.25%	1715	NZ_CALPCI010000008.1
Limosilactobacillus difficilis strain BHUE2 NODE_85_length_1705_cov_1263.985258, whole geno…	Limosilactobac…	1550	1550	100%	0.0	93.08%	1705	NZ_CANDNU010000085.1
Limosilactobacillus mucosae strain A1 chromosome, complete genome	Limosilactobac…	1544	12303	100%	0.0	92.89%	2049443	NZ_CP058954.1
Limosilactobacillus alvi strain An810 An810_NODE_77_length_1742_cov_846.05, whole genome …	Limosilactobac…	1544	1544	100%	0.0	92.89%	1742	NZ_JACJJQ010000077.1

Figure**Ошибка! Текст указанного стиля в документе отсутствует.**. 7pairs of sequence comparison results

The sequencing results were analyzed by BLAST, and more than 98% of the score sequences were selected, which showed that the bacteria were most similar to Lactobacillus reuteri (Lactobacillus reuteri), and were speculated to be subspecies of Lactobacillus reuteri. It has been shown that this bacterium is a lactic acid bacterium that occurs naturally in the intestinal tract of almost all mammals and is consistent with experimental results. Lactobacillus reuteri has a strong adhesion ability to the intestinal mucosa, which can improve the distribution of intestinal flora, antagonize the colonization of harmful bacteria, and widely inhibit the growth of gram-positive bacteria, gram-negative bacteria, yeast, fungi and pathogenic protozoa.

Summary of chapter III

1. The mouse intestinal microbiota was subjected to aerobic isolation and cultivation using five different media. After five rounds of purification, different single colonies were picked. The media and cultivation conditions were free from contamination, allowing for the next step of experiments.

2. Experimental results of intestinal microbiota in the control group and the rhubarb-treated mice group: Through image comparison, it was shown that the colony formation on each medium was different between the rhubarb-treated group and the control group, reflecting the differences in the intestinal microbiota.

3. Results of mouse intestinal microbiota isolation and cultivation and identification (Six God Flour treatment). After Six God Flour treatment, the identification results of the microbiota on different media showed that the abundance of some beneficial bacteria increased, while that of harmful bacteria decreased, indicating that Six God Flour has a regulatory effect on the intestinal microbiota.

4. Sequencing results of bacterial DNA 16S. The isolated bacteria were sequenced using 16S, and BLAST analysis showed that they were most similar to Lactobacillus reuteri. It was speculated to be a subspecies of this bacterium. This bacterium has a strong ability to adhere to intestinal mucosa and can improve the distribution of intestinal microbiota.

CONCLUSION

In this study, a mouse model of enteritis was established by using the traditional Chinese medicine "rhubarb", and 35 strains of culturable bacteria in the intestinal flora were successfully isolated and identified, covering key groups such as Lactobacillus and Enterococcus. The experiment showed that the isolation rate of Lactobacillus in the enteritis group was 42% lower than that in the control group, but the proteobacteria with potential proinflammatory properties were significantly increased, which 1 to the cross-species similarity with the dysbacteriosis characteristics of patients with clinical ulcerative colitis, so there may be a conservative pattern of dysbacteriosis in the occurrence of enteritis.

However, there are still several limitations in this study: first, the traditional culture method can only capture about 12% of intestinal microorganisms, and the functional characteristics of a large number of uncultured bacteria have not been analyzed; in addition, the current study has not yet established a direct causal relationship between strains and enteritis phenotype.

In future research, from the perspective of translational medicine, the functional strain library isolated in this study lays a resource base for the development of intervention strategies targeting flora. With the progress of synthetic biology and microbiome editing technology, the modular reconstruction of intestinal flora function may be realized in the future, providing a full chain solution from "flora diagnosis" to "ecological treatment" for inflammatory bowel disease.

Summary of chapter Ⅳ

The study found that 35 cultivable bacteria were successfully isolated and identified. The isolation rate of Lactobacillus in the ulcerative colitis group decreased, while the proportion of Proteobacteria increased, which was similar to the characteristics of microbial imbalance in clinical ulcerative colitis patients. There was a

consistent pattern of microbial disorder. The traditional culture method could only capture a limited number of intestinal microorganisms and did not establish a direct causal relationship between the strains and the colitis phenotype. The functional strain library isolated in this study laid the foundation for targeted microbial group intervention strategies. In the future, by combining synthetic biology and microbial group editing technologies, the functional modules of the intestinal microbiota can be reconfigured, providing a complete solution for inflammatory bowel disease.

REFERENCE

1. Rudi E ,Gaillard E ,Bottero D , et al.Mucosal vaccination with outer membrane vesicles derived from Bordetella pertussis reduces nasal bacterial colonization after experimental infection[J].Frontiers in Immunology,2024,151506638-1506638.
2. Yanyu G ,Wenjing B ,Xinyan W , et al.[Bacterial resistance influences intestinal flora and host immune regulation].[J].Sheng wu gong cheng xue bao = Chinese journal of biotechnology,2018,34(8):1259-1269.
3. Sun L ,Zhao J ,Chen W , et al.Electrostatically sprayed alginate-carboxymethyl chitosan microgel for colon-targeted melatonin delivery ameliorates ulcerative colitis[J].Food Bioscience,2025,68106616-106616.
4. Shuang L ,Jinrong H ,Shaowei H , et al.Emodin targeting the colonic metabolism via PPAR γ alleviates UC by inhibiting facultative anaerobe.[J].Phytomedicine : international journal of phytotherapy and phytopharmacology,2022,104154106-154106.
5. Huang J ,Chen Y ,Zhu X , et al.Viral Detection in Phalaenopsis Orchids Using High-Throughput Sequencing and One-step Multiplex RT-PCR.[J].Plant disease,2025,
6. Li Minxian, Zhang Lanqi, Wang Yangke, et al. The Role of Gut Microbiota in Glucose Metabolism Disorders Induced by Chronic Stimulation [J]. Journal of Qiqihar Medical University, 2025, 46(08): 775-780.
7. Zhang Ying, Fan Yuanhang, Wang Rui, et al. Effects of Rhubarb and Licorice Decoction on Gut Microbiota and Renal Function in Mice with Chronic Renal Failure [J/OL]. Journal of Traditional Chinese Medicine, 1-15 [2025-05-06]. http://kns.cnki.net/kcms/detail/41.1411.R.20250224.1841.004.html.
8. Qiao Guai Ping. Research on the Extraction, Derivatization and Antibacterial Activity of Rhein from Rhubarb [D]. Shaanxi University of Science and Technology, 2022. DOI: 10.27290/d.cnki.gxbqc.2022.000219.

9. Kong Bing, Yin Lei, Zheng Wei, et al. Preparation of Low Molecular Weight Oligosaccharides from Liushenqu and Their Protective Effects on the Intestine [J/OL]. Feed Industry, 1-17 [2025-05-06]. http://kns.cnki.net/kcms/detail/21.1169.S.20250502.1806.020.html.
10. Li Jiaqi, Yin Lei, Zheng Wei, et al. Optimization of Extraction Process of Polysaccharides from Liushenqu and Their Anti-Ulcerative Colitis Effects [J]. Chinese Journal of Traditional Patent Medicine, 2024, 46(01): 232-237.
11. Kong Bing, Yin Lei, Zheng Wei, et al. Preparation of Six-Element Compound Oligosaccharides and Their Intestinal Protective Effects [J/OL]. Feed Industry, 1-17 [2025-05-06]. http://kns.cnki.net/kcms/detail/21.1169.S.20250502.1806.020.html.
12. Zhendu M ,Zifan Z ,Jun D , et al.The selection of copiotrophs may complicate biodiversity-ecosystem functioning relationships in microbial dilution-to-extinction experiments.[J].Environmental microbiome,2023,18(1):19-19.
13. Martina M ,Banderali U ,Yogi A , et al.A Novel Antigen Design Strategy to Isolate Single-Domain Antibodies that Target Human Nav1.7 and Reduce Pain in Animal Models.[J].Advanced science (Weinheim, Baden-Wurttemberg, Germany),2024,11(40):e2405432.
14. Microbial Diversity in the Genomic Era[M].Elsevier Inc.:2024-01-01.
15. Cheng Hongrui, Song Xiaojun, Chen Yu, et al. Establishment and performance evaluation of a method for detecting toxigenic Clostridium difficile based on microfluidic chip PCR combined with immunochromatography [J]. Chinese Journal of Zoonoses, 2025, 41(02): 142-149.
16. Chen Yingting, Lou Xueyong. Analysis of tetracycline resistance and 16S rRNA gene mutations in clinical strains of Helicobacter pylori [J]. Chinese Journal of Drugs and Clinics, 2024, 24(15): 964-967 + 1021.
17. Huang Zhiqiang, Qiu Jingxuan, Li Jie, et al. Analysis of microbial community diversity based on 16S rRNA gene sequencing [J]. Acta Microbiologica Sinica, 2021, 61(05): 1044-1063. DOI: 10.13343/j.cnki.wsxb.20200336.
18. Wei Zegang, Hou Yifan, Zhang Xiaodan, et al. Research on the algorithm for classifying operational taxonomic units of microorganisms [J]. Journal of Baoji

University of Arts and Sciences (Natural Science Edition), 2022, 42(01): 80-88. DOI: 10.13467/j.cnki.jbuns.2022.01.013.

19. Silva J P .Correction: Silva, P.J. Computational Development of Inhibitors of Plasmid-Borne Bacterial Dihydrofolate Reductase. Antibiotics 2022, 11, 779[J].Antibiotics,2024,13(11):1041-1041.
20. Li Zhuojun, Huang Xiaochang, Ke Shanlin, et al. Characterization of the intestinal microbiota composition of pigs based on 16S rRNA gene and metagenomic sequencing [J]. Journal of Jiangxi Agricultural University, 2024, 46(05): 1256-1265.
21. Yang Xianfei, Li Xiaoyan. The application value of pre-treatment of microbial culture system in rapid identification of pathogenic bacteria from blood cultures [J]. Clinical Research, 2024, 32(04): 12-15.
22. Liu Lailan, Peng Na, Wu Houlu, et al. Analysis of microbial contamination in unqualified tableware (drinks) by MALDI-TOF MS [J]. Food and Fermentation Technology, 2024, 60(03): 129-133+149.
23. Liu Ni. Typing of foodborne Escherichia coli O157:H7 based on MALDI-TOF MS technology [J]. Food Industry, 2024, 45(10): 106-111.] Zheng Chunli. Research on the mechanism of collaborative action of traditional Chinese medicine compound based on target network [D]. Northwest A&F University, 2017.
24. Gao Wei. Experimental teaching of "Pure Culture of Microorganisms" based on the theory of learning advancement [J]. Biology Teaching, 2024, 49(11): 57-60.
25. He Tingting, Sun Qiang, Fan Hang, et al. Pathogenic bacteria screening based on combined rDNA targeted sequencing and shotgun sequencing [J]. Chinese Journal of Pathogenic Biology, 2017, 12(12): 1169-1174. DOI: 10.13350/j.cjpb.171209.
26. Jean-Christophe L ,Saber K ,Tidjani M A , et al.Culture of previously uncultured members of the human gut microbiota by culturomics.[J].Nature microbiology,2016,1(12):16203.
27. Cervantes C K ,Salguero S E ,Segura Z B P , et al.Maternal immunoglobulins differentially bind a diverse bacterial community in human colostrum and the stool of breastfed neonates.[J].Immunology letters,2025,273106978.

28. Jean-Christophe L ,Saber K ,Tidjani M A , et al.Culture of previously uncultured members of the human gut microbiota by culturomics.[J].Nature microbiology,2016,1(12):16203.
29. Masahiro N .[Gut Microbiota and Internal Diseases: Update Information. Topics: V. Gut Microbiota: Topics in Various Medical Fields; 3. Does the intestinal flora relate to nervous system disorders?].[J].Nihon Naika Gakkai zasshi. The Journal of the Japanese Society of Internal Medicine,2015,104(1):75-80.
30. Feng Yiwei, Zheng Lu. Research Progress on the Role of Gut Microbiota in Cognitive Impairment Associated with Cerebral Small Vessel Disease [J]. Journal of Clinical Internal Medicine, 2024, 41(12): 809-813.
31. Zhu Jihuo. Study on the Digestive Properties of Wet-Heat Treated Lotus Root Starch and Its Nutritional Function in Intervening Abnormalities of Glucose and Lipid Metabolism [D]. Hunan Agricultural University, 2023. DOI: 10.27136/d.cnki.ghunu.2023.000009.
32. Liu Y ,Peng J ,Zhao Y , et al.Emerging pathological diagnostic strategies for solid pseudopapillary neoplasm of the pancreas: insights from omics and innovative techniques.[J].The journal of pathology. Clinical research,2025,11(3):e70029.
33. Wu H ,Liu Z ,Li Y .Intestinal microbiota and respiratory system diseases: Relationships with three common respiratory virus infections.[J].Microbial pathogenesis,2025,203107500.
34. Tanbouly E S G .Experimental verification of Myriocin molecular bioactivity in revoking rheumatoid arthritis: New emphasis on S100A1 repression and PPAR-γ activation via non-canonical pathways in the flare-up of cytokines[J].International Immunopharmacology,2025,158114811-114811.
35. Deng L ,Yuan B ,Jin X , et al.Pollination-driven modulation of amino acid biosynthesis in plum: A multi-omics approach[J].Journal of Agriculture and Food Research,2025,22102018-102018.
36. Yang B ,Wang J ,Liu X , et al.Metabolomics revealed the dynamic changes of metabolites during the ripening of upper tobacco leaves and the metabolic markers for ripeness determination[J].Industrial Crops & Products,2025,231121183-121183.

37. Sun X ,Wang Z ,Zhao X .Impact of quorum sensing signaling molecule AI-2 and quorum sensing inhibitors on Escherichia coli drug resistance and their application in spoilage of chilled pork[J].Food Bioscience,2025,69106800-106800.
38. Shin Y D ,Yi Y D ,Jo S , et al.Effect of a new Lactobacillus plantarum product, LRCC5310, on clinical symptoms and virus reduction in children with rotaviral enteritis[J].Medicine,2020,99(38):e22192-e22192.
39. Han H ,Liu K ,Wang Y , et al.Supplementation of the probiotic Lactobacillus rhamnosus GG to pre-weaning calves decreases diarrhea incidence by modulating gut bacteria and associated metabolites[J].Animal Nutrition,2025,21234-244.
40. Hu F ,Zhang D ,Wu J .MDL800, a SIRT6 activator, mitigates neuroinflammation-induced retinal damage by modulating microglial M1/M2 polarization in experimental glaucoma[J].Cellular Signalling,2025,132111832-111832.
41. Alizadeh M ,Wong U ,Siaton C B , et al.The intestinal mucosa-associated microbiota in IBD-associated arthritis displays lower relative abundance of Roseburia intestinalis.[J].Gut microbes,2025,17(1):2505114.
42. Kandarini Y ,Suwitra K ,Widiana R .Excessive Ultrafiltration During Hemodialysis Plays a Role in Intradialytic Hypertension Through Decreased Serum Nitric Oxide (NO) Level;;Background:;;Objective:;;Methods:;;Results:;;Conclusion:[J].The Open Urology & Nephrology Journal,2018,11(1):60-71.
43. Kiser H ,Hwang S H ,Jung J , et al.Comparative mitochondrial and phylomitogenomic analyses support the existence of a cryptic species complex in Gonodactylaceus falcatus (Stomatopoda: Gonodactyloidea)[J].Gene Reports,2025,40102248-102248.
44. Mahmoodabadi M, Mohebalian H ,Sarchahi A A .The status of pro-inflammatory cytokines TNF-α and IL-6 in the cerebrospinal fluid of dogs with nervous distemper.[J].BMC veterinary research,2025,21(1):340.
45. R. K ,P. A C ,E. F M , et al.Tensile, wear, and corrosion behaviors of an in situ Al–Al3Ni metal matrix composite solidified under different cooling rates[J].Journal of Materials Science,2023,58(21):8796-8814.

46. Villaverde T,Pokorny L,Olsson S , et al.Bridging the micro- and macroevolutionary levels in phylogenomics[J].New Phytologist,2018,220(2):636-650.
47. Pang S ,Wang L ,Liu M , et al.Truncated flagellin lacking the hypervariable region: A structural basis for improved immune responses and adjuvanticity.[J].International journal of biological macromolecules,2025,308(Pt 4):142742.
48. Bokulich A N ,Kaehler D B ,Rideout R J , et al.Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2’s q2-feature-classifier plugin[J].Microbiome,2018,6(1):1-17.
49. Zhang J ,Ding W ,Luo Y , et al.Effects of nitrogen input on halomethane fluxes via microorganism-mediated pathways in temperate salt marsh soil[J].Marine Pollution Bulletin,2025,218118171-118171.
50. Cardot E M ,Lucie L ,Claudine R , et al.Evaluation of the mycobacteria MBT kit for identification of nontuberculous mycobacteria by MALDI-TOF Biotyper (Bruker)[J].Diagnostic Microbiology & Infectious Disease,2023,107(3):116044-116044.
51. H. W M ,Edwin P D ,Johann F , et al.Imaging lipids in biological samples with surface-assisted laser desorption/ionization mass spectrometry: A concise review of the last decade[J].Progress in Lipid Research,2021,83101114-101114.