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Dissection of the key intestinal microbiota in residents of Pemba Island in Tanzania and use of *Bifidobacterium breve* in prevention against colitis in mice

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To my beloved parents and tutor

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CHAPTER I GENERAL INTRODUCTION

1.1 GUT MICROBIOTA COMPOSITION

Gut microbiota is a complex community of microorganisms that inhibit in the gastrointestinal (GI) tract of humans and animals. More than 100,000 billion microorganisms live in the GI tract including bacteria, viruses and some eukaryotes, which is 10 ~ 100 times the number of human cells[1]. The gut microbiota could encode over 3 million genes producing thousands of metabolites, which is approximately 150 times the number of human genomes. The tremendous number of microbes and microbiome genes determine that gut microbiota could adapt to the complicated and flexible environments and constitute a mutually beneficial symbiotic relationship with the host[2, 3]. As a "superorganism", gut microbiota plays a significant role in host health. Commensal bacteria contribute to nutrient absorption, metabolite regulation, enteric pathogen colonization, and immune system development[4, 5]. Previous studies have confirmed that intestinal homeostasis is associated with the health status of the individuals. And intestinal dysbiosis are associated with chronic problems, such as inflammatory bowel disease (IBD), irritable bowel syndrome, obesity, diabetes and other metabolic diseases, allergic diseases, neurodevelopmental diseases and parasitic infections[6-10]. Supplement with probiotics, prebiotics or fecal microbiota transplantation (FMT) are considered effective approaches to relieve the diseases caused by gut microbiota dysregulation[11, 12]. Therefore, to reshape the gut microbial community as an alternative therapy for the treatment of certain diseases, has been a hot spot of scientific research in recent years to prompt human health.

Domain, phyla, classes, orders, families, genera, and species are commonly used in bacterial taxonomy. The intestinal microbiota harbors 1000 ~ 1500 bacterial species within more than 50 phyla[13]. The composition of human gut microbial community is relatively fixed, mainly followed by Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, Tenericutes and Verrucomicrobia at phylum level, possessing about 90% of the total bacterial population[14]. Usually, Firmicutes take up

the largest percentage of the total bacteria. Besides, the abundance of Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, Tenericutes and Verrucomicrobia decreased successively in the gut[15]. Each bacterial phylum has its typical representative genera. *Lactobacillus*, *Faecalibacterium*, *Ruminococcus*, *Roseburia*, and *Clostridium* are representative genera belonging to Firmicutes[16]. *Bacteroides*, *Prevotella* and *Alistipes* are representative genera of Bacteroidetes[17]. The Actinobacteria phylum is mainly represented by *Bifidobacterium* and *Collinsella*. *Akkermansia muciniphila* is the only member of Verrucomicrobia[18-20]. The intestinal flora of healthy individuals showed a high level of consistency in the composition at phylum level. While there are large differences between individuals in the gut composition at bacterial genus level. In recent years, intensive investigations were carried out on dissections of the gut microbial structure, elucidations on the specific function and metabolic pathway of gut microbiota.

The development of high-throughput sequencing technology and bioinformatics technology has enabled researchers to excavate deeper information from studies on intestinal microbes and make greater breakthrough. Human microbiome project (HMP) is an extension and supplement of the Human Genome Project, and the research on gut microbiota from different countries and nations around the world has been reported one after another[21]. The concept of "Enterotype" was put forward in the year of 2011. An enterotype is a classification of living organisms based on the bacteriological composition of their gut microbiota[22]. Three enterotypes have been reported so far: *Bacteroides* (enterotype I), *Prevotella* (enterotype II) and *Ruminococcus* (enterotype III). *Bacteroides* has been associated with a Western food diet and it is the predominant genus with the highest abundance in the GI tract of individuals with a Western food diet. *Prevotella* has been associated with an agrarian-type diet and it is the top genus with the highest abundance in the gut of individuals from African agricultural societies or hunter tribe. *Ruminococcus* is the most abundant bacteria at the genus level in the GI tract of Taiwanese[22-24]. Studies indicate that enterotypes are not dictated by age, gender, body weight, or national divisions, while are only influenced by long-term dietary

pattern[25]. *Bacteroides* enterotype is strongly related with a high-calorie, high-fat and high-protein diet. And *Prevotella* enterotype is associated with a diet mainly based on plant polysaccharides and fiber.

1.2 FUNCTIONS OF THE GUT MICROBIOTA

1.2.1 Role of gut microbiota in metabolism

Gut microbiota plays a key role in the secretion, synthesis, and absorption of many nutrients and metabolites including carbohydrates, proteins, lipids, vitamins, polyphenols, bile acids, and short-chain fatty acids (SCFAs)[26]. The various metabolic products generated by gut microbiota affects human health and the microbial metabolic pathways are intensively investigated at present.

Bacteria in the gut could digest and convert dietary components to bioactive substances. The intestine-colonized bacteria could metabolize indigestible carbohydrates like cellulose, hemicelluloses, resistant starch, pectin and oligosaccharides into short chain fatty acids (SCFAs) including acetate, propionate and butyric butyrate[1]. SCFAs is able to provide energy for epithelial cells and maintain the integrity of epithelium to keep gut homeostasis[27]. SCFAs-producing bacteria are mainly members belonging to Firmicutes and Bacteroidetes. The deficiency of the SCFAs-producing bacteria is usually associated with intestinal disorders in the host[28].

Gut microbiota plays a key role in the synthesis of vitamin such as vitamin B, vitamin K, biotin, thiamine, nicotine, pantothenic acids, cobalamin and riboflavin, by contributing enzymes which are not encoded by the human genome. *Bacteroides fragilis*, *Eubacteirium lentum*, *Enterobacter agglomerans*, *Serratia marcescens* and *Enterococcus faecium* are involved in the synthesis of vitamin B and K[29-31]. Moreover, the biosynthesis of riboflavin, biotin and pantothenic acids are mainly involved in the individual with the enterotype of *Bacteroides*, while the biosynthesis of thiamine is mainly involved in the individual with the enterotype of *Prevotella*[32].

Gut microbiota have been demonstrated to synthesize some neurochemicals, such as dopamine, norepinephrine, serotonin, and gamma-aminobutyric acid (GABA) to

affect the central nervous system[33]. Manipulation of the neurotransmitters produced by bacteria may have a positive impact on host physiology. GABA is an important inhibitory neurotransmitter and involved in regulating the process of neurological function. *Bifidobacterium* and *Lactobacillus* have been reported to produce GABA to reduce depressive- and anxiety-like behavior in the mice model[34]. The mechanism of the modulation of GABA produced by the microbiota on neuropsychiatric disorders needs to be elucidated in the future.

Bile acids, phenols, indole metabolites, antimicrobial peptides are also are produced by gut microbiota[35]. Indole metabolites (indole, indole-3 propionic acid, etc.) play an important role in maintaining the intestinal barrier function and in prevention from viruses and pathogenic bacteria entering the blood. The microbiota could metabolize amino acids to generate various bioactive signal molecules with small molecular mass and antimicrobial peptides. The bioactive signal molecules effectively regulate the functions of important organs in humans such as the cognitive function of the brain, and the metabolic functions of the liver and pancreas[14].

1.2.2 Protective role of gut microbiota

Gut microbiota could inhibit pathogen infections by directly competing for the limited space of habitats and limited resource of nutrients in the intestine[36]. Bacteria in the gut also enhance mucosal barrier function to prevent the adhesion of pathogens to the intestinal epithelium[37]. Besides, antimicrobial molecules, SCFAs, and hydrogen peroxide are produced in the actions of gut microbiota to prevent the invasion of pathogens[38, 39]. SCFAs could serve as important energy source for intestinal epithelial cells and enhance epithelial defense[40]. What's more, butyrate has potential anti-inflammatory and anti-cancer properties to induce apoptosis of colon cancer cells and regulate gene expression by inhibiting histone deacetylases[41]. Most butyrate producers are from Lachnospiraceae belonging to Firmicutes in the GI. SCFAs-producing bacteria have lower amount in patients with colon cancer than in the healthy individual[42].

1.2.3 Microbiota and the gut–brain axis

The gut brain microbiota axis (gut-brain axis) is defined as a bidirectional

communication channel between the central nervous system and the enteric nervous system to achieve a cross talk[43]. The functions of the gut brain axis are to monitor and regulate gut functions, and link emotional and cognitive centers of the brain with peripheral intestinal functions, including immune activation, intestinal permeability, enteric reflex, and entero-endocrine signaling[44]. Previous studies have revealed that intestinal microbial colonization is important to the development and maturation of central nervous system[45]. The deficiency of some bacterial colonization is related to an altered level of neurotransmitters in the human central and enteric nervous system[46]. Gut microbiota could produce SCFAs to maintain the integrity of the blood brain barrier which prevents harmful metabolites into brain tissue[47]. Moreover, lipoprotein and lipopolysaccharide produced by gut microbiota could affect autoimmune function by expressing cytokines which are able to cross blood brain barrier to activate neurons[48].

1.2.4 Microbiota and the gut–liver axis

The gut-liver axis refers to the bidirectional relationship between the intestine along with the intestinal microbiota, and the liver, resulting from the integration of signals generated by dietary, genetic and environmental factors[49]. Studies have shown that intimate cross talks occur between the gut and the liver[50]. When the intestinal barrier is damaged with an increased permeability, the liver is automatically exposed to numerous toxic substances derived from the intestinal bacteria[51]. Recent research indicates that changes of microbial composition play a vital role in the development of nonalcoholic fatty liver disease (NAFLD)[52]. An increased level of Bacteroidetes, a decreased level of Firmicutes, and a larger proportion of pro-inflammatory taxa such as Proteobacteria and Enterobacteriaceae were observed in individuals with NAFLD than in the healthy individuals[53].

1.3 FACTORS AFFECTING GUT MICROBIAL COMPOSITION

1.3.1 Diet

Diet is a central modulator which influences the composition of the gut microbiota. Short-term dramatic food dietary pattern is reported to alter gut microbial diversity fast

and long-term food dietary pattern contributes to form stable microbiota profile in humans[25, 54]. Delivery method and breast feeding are the key factors affecting the neonatal gut microbiota after birth. Later in the infant, digestion of solid foods brings huge changes in the intestinal community. Higher abundances of Actinobacteria and lower abundances of Firmicutes and Proteobacteria are in the gut of breast-fed infants, while higher amounts of *Clostridia*, *Streptococci*, *Bacteroides* and *Enterbacteria* are in the gut of formula-milk fed infants[55, 56]. After infancy, the gut microbiota continues its development, and the habitual diet plays an integral role in shaping the gut microbiota. Western pattern diet, which is a typical animal-based diet, is usually associated with higher abundances of *Bacteroides*, *Alistipes* and *Bilophila*, and lower abundances of *Lactobacillus* and *Roseburia*, manifesting as a decreased intestinal microbial diversity. Western diet has been connected to chronic low-grade inflammation and metabolic disease[57, 58]. Vegetarian pattern diet is a plant-based diet and is associated with higher amount of *Prevotella*, *Faecalibacterium* and *Lactobacillus*. Moreover, vegans or vegetarians usually have a significantly greater bacterial alpha diversity in the gut. Vegetarian diet has been linked to health[59]. Mediterranean diet is mainly based on plant, high in fiber and low in animal protein. Higher abundances of *Lactobacillus*, *Clostridium*, *Faecalibacterium*, and *Oscillospira*, and a lower abundance of *Ruminococcus* and *Coprococcus* were observed in the participants with a Mediterranean diet[60]. At the same time, adherence to the Mediterranean diet is positively associated with the production of SCFAs and secretion of anti-inflammatory cytokines to reduce the risk of chronic inflammatory diseases[61].

1.3.2 Age

It has been revealed by several studies that age affects the human gut microbiota. Vaginal or cesarean delivery is the key factor to decide the gut composition of the newborn infant. Exposed to different environments, the colonization of the microbes is destined to be different at the beginning. After birth, the first colonizer in the gut are aerobic bacteria from Proteobacteria. Then the aerobic bacteria consume oxygen in the gut allowing anaerobic bacteria to colonize[62]. At the age of 1 ~ 2 years, this window

is critical for the development of the gut microbiota, the infant is subject to low microbial diversity at first, then increased over time[63]. Around the age of 2 ~ 5 years, the gut microbiota composition in childhood becomes stable and towards a more diverse adult-like microbiota. Increased kinds and numbers of butyrate-producing bacteria belonging to Firmicutes and Bacteroidetes are observed in this period[63]. In pre- adolescent (7 ~ 12 years), the gut microbiota was reported to be enriched in some bacteria which could synthesize folic acid and vitamin B12 to promote the continuous physical development of the host[64]. In the adolescent (11 ~ 18 years), the levels of *Clostridium* and *Bifidobacterium* were significantly higher than those in adults[65]. In healthy adults, the composition of the gut microbiota is relatively stable, and the stability can maintain for decades. The gut composition of healthy adults is mainly followed by Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, Tenericutes and Verrucomicrobia[66]. Aging significantly affects the function of gut microbiota, especially after the age of 65. The aging of teeth, the decline of salivary secretion, and the decline of gastrointestinal digestion and absorption ability, are all reported to lead to the reduction of the intestinal bacterial diversity, the expansion of opportunistic pathogens, and a decrease in probiotics and SCFAs-producing bacteria in the elderly people[67]. Compared with the adults, the gut microbiota of the elderly people showed a lower ratio of Firmicutes to Bacteroidetes and a lower abundance of Enterobacteriaceae[68].

1.3.3 Host genetics

Genetics significantly influence the diversity of the gut microbiota and susceptibility to pathogenic microorganisms in healthy individuals[69]. Research has shown that monozygotic twins who lived apart for many years showed a high degree of similarity in the composition of their gut microbiota, while marital partners who live in the same environment and have similar diet didn’t show the results like the monozygotic twins[63]. High similarity of heritable taxa and functional categories of candidate genes among pig, human and mouse were confirmed. And Firmicutes, Actinobacteria, Tenericutes, and Euryarchaeota were reported to be more heritable,

while Bacteroidetes exerted very little heritability[70, 71].

1.3.4 Exercise

Daily exercise could increase gut microbial diversity with a higher abundance of SCFAs-producing bacteria such as Clostridiales, Roseburia and Lachnospiraceae which up-regulates the expression of tight junction proteins to enhance the intestinal barrier function, to reduce mucosal permeability and inhibit inflammatory cytokines secretion. Multiple studies have indicated that exercise is positively associated with protein intake and creatine kinase levels[72]. Higher levels of Firmicutes and lower levels of Bacteroidetes are observed in athletes than non-mobilized individuals[73]. Moreover, exercise is positively associated with the abundance of *Lactobacillus*, *Bifidobacterium*, and *Akkermansia* in the gut and negatively associated with Proteobacteria, *Turicibacter*, and Rikenellaceae[71].

1.3.5 Antibiotics

Antibiotic treatment could eliminate pathogenic and beneficial intestinal bacteria at the same reducing the diversity of microbial species, causing gut dysbiosis, decreasing the competitive exclusion and stimulating the development of bacterial antibiotic resistance[74]. A decreased level of *Bifidobacterium* and increased level of Enterobacteriaceae was detected in the gut of adult individuals after treatment of a combination of meropenem, gentamicin, and vancomycin for a period time[75]. Clarithromycin treatment causes a decline in the abundance of actinobacteria in the patients with *Helicobacter pylori* infection[76]. Antibiotic exposure also produces changes in the gut metabolome. Cho et al. reported that low-dose antibiotics led to increased adiposity and enhanced hormones associated with the metabolism of carbohydrates, lipids and cholesterol[77].

1.3.6 Smoking

Cigarette smoke is a complex chemical compound including toxic ingredients. The toxicants of cigarette smoke swallowed into the gastrointestinal tract induce intestinal inflammation and dysbiosis[78]. Clinical studies revealed that patients with chronic lung diseases have a higher prevalence of intestinal diseases, such IBD and IBS, and lung

diseases may be implicated in intestinal diseases through lung-gut axis[79]. Significant higher abundance of Firmicutes and Actinobacteria, lower abundance of Bacteroidetes and Proteobacteria were observed in the gut of smokers than the non-smokers[80]. Besides, smoking has been confirmed to alter the bacterial community in the oral cavity as well[81].

1.4 RELATIONSHIPS OF GUT MICROBIOTA WITH DISEASES

1.4.1 Gastrointestinal diseases

1.4.1.1 Inflammatory bowel disease (IBD)

IBD is one of the most common GI disorders nowadays including ulcerative colitis (UC) and Crohn's disease (CD). Genetic and environmental factors such as stress, sleep, antibiotic use, diet and smoking, are involved in the pathogenesis of the intestine disease[82]. Gut dysbiosis is mainly responsible for the develop of IBD. The loss of bacteria belonging to Firmicutes would jeopardize the mucosal immune response and disrupt regulatory functions of T lymphocytes to inducing intestinal inflammation to develop IBD[83, 84]. Gut microbiota variations are reported in the gut composition of patient individuals with IBD. Decreased abundance of the butyrate-producing bacteria such as *Roseburia hominis* and *Faecalibacterium prausnitzii*, and increased abundance of *Escherichia* are observed in UC patients than healthy individuals[85, 86]. Decreased levels of *Dialister invisus*, *Clostridium*, *Faecalibacterium prausnitzii*, and *Bifidobacterium adolescentis*, increased levels of *Neisseriacaea corrodens*, *Veillonella parvula* and *E. coli* are observed in CD patients than controls[7, 87]. Moreover, some species of yeast and fungal such as *Cyberlindnera jadinii*, *Calvispora lusitaniae*, *Candida albicans*, *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* also showed higher amount in patients with CD[88].

1.4.1.2 Irritable bowel syndrome (IBS)

Irritable bowel syndrome (IBS) is a functional disease characterized by abdominal pain, flatulence and altered bowel habits. Studies have revealed that gut disorders are involved in IBS pathogenesis through facilitating the adhesion of pathogens to the

bowel wall[89]. Enrichment of Firmicutes (*Ruminococcus*, *Clostridium* and *Dorea*) and Proteobacteria (Enterobacteriaceae), and reduction of Bacteroidetes and Actinobacteria are in the gut of adult IBS patients than in the normal controls[90]. Pediatric IBS patients are reported to have the similar alterations amongst the members of Firmicutes and Proteobacteria like adult IBS patients[91, 92].

1.4.1.3 Colorectal cancer (CRC)

Colorectal cancer is the third leading cause of cancer-related deaths in humans around the world[93]. Significant differences of gut microbiota structure between CRC patients and healthy individuals have been demonstrated[94]. Increased abundance of *Bacteroides fragilis*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Streptococcus*, and *Peptostreptococcus* were shown in the CRC patients, and reduction of butyrate producers (Lachnospiraceae and *Roseburia*) were in the CRC patients[94]. Shen et al. also reported that higher abundances of *Dorea* and *Faecalibacterium*, lower abundances of *Bacteroides* and *Coprococcus* in CRC patients compared with healthy controls[95]. Alterations in gut microbiota of CRC patients may contribute to the etiology of colorectal cancer. To manipulate microbiota as a novel therapy to prevent colorectal cancer is full of interest in the further research.

1.4.2 Hepatic diseases

1.4.2.1 Non-alcoholic fatty liver disease (NAFLD)

NAFLD has become the most common liver disease worldwide. Fat accumulation in the hepatocytes is the classical symptom of NAFLD. Dysbiosis, diet, changes of intestinal permeability, genetics are all factors that affect NAFLD develop[96]. Research have indicated that altered intestinal bacterial community (lower abundance of Firmicutes; higher abundance of Bacteroidetes) and increased intestinal permeability are observed in patients with NAFLD compared with the healthy controls[97]. The decrease in ratio of Bacteroidetes to Firmicutes elevates a higher energy harvest that could initiate gluconeogenesis and lipogenesis in the liver[98].

1.4.3 Metabolic diseases

1.4.3.1 Obesity

Obesity is a multifactorial disease resulting in excessive accumulation of fat tissue. Obesity is highly prevalent around the world with a number of 700 million over-weight individuals in 2015, and the number is still increasing. Except genetics, diet, lifestyle and other environmental factors, gut microbiota also plays an important role in the pathogenesis of obesity[99]. Obesity is associated with alterations in the intestinal ratio of Firmicutes to Bacteroidetes in the obese people[100]. Moreover, a high proportion of Firmicutes to *Bacteroides*/*Prevotella* in obese people enhances the microbial genes involved with the degradation of polysaccharide and promotes the generation of SCFAs[101]. And higher levels of *Campylobacter*, *Bacteroides*, *Staphylococcus*, *Parabacteroides*, *Dialister* and *Ruminococcus* are detected in the obese subjects than the lean subjects, and lower levels of *Lactobacillus*, *Bifidobacterium*, *Faecalibacterium*, *Akkermansia*, *Methanobrevibacter* and *Coprococcus* are determined in the obese subjects too[102].

1.4.3.2 Diabetes

Type I diabetes is a chronic disease resulting from the destruction of pancreatic β cells that produce insulin. Several studies have confirmed that type 1 diabetes is associated with a high abundance of Bacteroidetes, and a deficiency of lactate and butyrate producing bacteria[103]. Type II diabetes (T2D) is a chronic metabolic disorder where the body either does not produce enough insulin. It has been confirmed that gut microbial composition is altered in patients suffering from T2D. The ratio of Bacteroidetes to Firmicutes, and the ratio of *Bacteroides* to *Prevotella*, are both highly increased in T2D sufferers compared to healthy patients. What's more, gut microbiota regulates polysaccharides and energy metabolism through the production of SCFAs[104]. Butyrate exerts beneficial effect on insulin sensitivity by blocking translocation of endotoxic compounds derived from the gut microbiota to drive insulin resistance[105].

1.4.4 Cardiovascular disease (CVD)

CVD is still the leading cause of death and disability in developed countries. Obesity, T2D and the metabolic syndrome are the conventual risk factors for CVD[106]. Trimethylamine (TMA) is an organic compound produced by the gut microbiota and

trimethylamine-N-oxide (TMAO) is the hepatic oxidation product of TMA. TMAO has gained much attention as a promising promoter of atherosclerosis and cardiometabolic diseases. In addition, TMAO have an impact on the metabolism of bile acid in the liver, such as suppression of bile acid synthetic enzymes and cholesterol transporters[107]. Reduced levels of *Roseburia* have been shown in the gut of atherosclerosis patients[108].

1.4.5 Allergic diseases

Allergies are global health issues influencing over half a billion people worldwide. Food allergy, allergic rhinitis, eczema, rheumatoid arthritis, and asthma are the common allergic diseases. Genetics, environment and gut microbiota are all the risk factors for allergies. Gut microbiota is considered to play a key role in regulating the severity of allergic diseases[109]. Research has indicated that reduced gut microbial diversity is associated with the increased risk of food allergy in school children[110]. In addition, studies also have shown that deletion of bacteria such as *Bifidobacterium*, *Akkermansia*, and *Faecalibacterium* in neonates may increase allergy risk by inducing T cell differentiation[10]. Reduced abundance of *Lachnospira*, *Veillonella*, *Faecalibacterium* and *Rothia* have been reported with increased risk of asthma in the infants during the first 100 days of life[9]. Several research also have indicated that dysbiosis plays a crucial role in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, T1D and CD[111-113].

1.4.6 Neurologic and psychiatric diseases

1.4.6.1 Autistic spectrum disorder (ASD)

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and behavioral impairment. Several studies have indicated that reduced microbial diversity, lower abundance of *Bifidobacterium*, and higher abundance of *Lactobacillus*, *Clostridium*, *Bacteroidetes*, *Desulfovibrio*, *Caloramator*, and *Sarcina* are observed in children with ASD than the normal children without ASD[114-117]. Wang et al. also reported a decreased abundance of *Bifidobacterium* and *Akkermansia muciniphila* in the gut microbiota of autism children compared with healthy subjects[118].

1.4.6.2 Alzheimer's disease (AD)

Alzheimer disease (AD) is a neurodegenerative disorder associated with progressive and degenerative impairment of behavioral and cognitive functions[119]. AD is the main cause for dementia in the elder people and contributes around 70 ~ 80% the dementia cases around the world[120]. Gut microbiota has been demonstrated to play important roles in the pathogenesis of AD[121]. Increased abundance of *Escherichia* is possibly related with a peripheral inflammation in patients with cognitive impairment and brain amyloidosis[122]. The gut microbiota in AD mice model exerted higher abundance of Bacteroidetes and Firmicutes, and lower abundance of *Akkermansia* and *Allobaculum* compared with those in the healthy mice model, respectively[123].

1.5 MICROBIOME-BASED THERAPEUTICS

1.5.1 Probiotic and prebiotic

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"[124]. Traditional probiotics consist of *Bifidobacterium* and *Lactobacillus*, and yeast *Saccharomyces boulardii*[125]. Probiotics could colonize and proliferate within the gastrointestinal tract shaping the gut composition. Probiotics are able to enhance gut epithelial barrier, increase mucus secretion, reduce infection or inflammation and regulate gastrointestinal permeability[126]. In addition, probiotics could improve nutrient absorption and utilization, energy harvest, hormone production, cognition and mood[127]. Different strains, doses and duration of administration of probiotics usually display different beneficial effects[128].

Probiotics could be used to prevent and treat various diseases, such as diarrhea, obesity, urinary tract infection, IBS, and diabetes[129]. Probiotics maybe also employed to inhibit tumors because its ability to combine with carcinogenic mutagens, inhibit mutation, and block the transformation of carcinogens. Probiotics reduce intestinal pH by producing SCFA, and secrete anti-inflammatory molecules to enhance the level of innate immune response[130]. Research have revealed that probiotic could serve as a

novel therapeutic method to relieve neurological and psychiatric diseases. Supplementation with probiotics such as *Lactobacillus*, *Bifidobacterium*, *Lactococcus* and *Streptococcus* could significantly improve psychological symptoms of depression, stress and anxiety[131]. The reduction of pro-inflammatory cytokines and improvement of neurotransmitter function may be involved in the mechanism[132].

A prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit"[133]. Cellulose, oligosaccharides, lignin and chicory root are common prebiotics and have been confirmed to be resistant with gastric acid, but not degraded by digestive enzymes. Prebiotics could be fermented by gut microbiota to promote the growth of some certain bacteria and to provide benefits to the host, such as maintaining intestinal mucosal barrier integrity, enhancing host mucosal immunity, lowering pH, stimulating SCFA production, and inhibiting the growth of pathogenic microorganisms[134, 135].

1.5.2 Fecal microbiota transplantation (FMT)

Fecal microbiota transplantation (FMT) is the infusion of a solution of fecal composition from a donor into the intestinal tract of a recipient in order to directly change the recipient's intestinal bacterial composition to cure a specific disease. Intensive studies have shown that FMT is the optimal choice for the treatment of IBD and *Clostridium difficile* infection by supplementing the microbiota instability through enhancing the abundance of Firmicutes and reducing Actinobacteria and Enterobacteriaceae, and FMT is able to rebuild the patient's gut microbiota composition and function similar to that of the donor[136]. In addition, FMT has also been applied to treat other diseases, such as IBS, Parkinson's disease, allergic disorders, fibromyalgia, chronic fatigue syndrome, myoclonus dystopia, multiple sclerosis, obesity, insulin resistance, metabolic syndrome, and autism[137].

The mechanism of therapeutic FMT on the treatment is not clear. And alterations in intestinal microbial composition, changes of host metabolic profiles and modulation of gut microbes from the healthy donors are possible mechanisms[138]. There are two main resources supplying fecal samples for FMT: patient selected donor and stool bank.

The patient-selected donor refers as, the patient or their guardian identifies their own stool donor candidate. The stool bank is a centralized facility that screens donors, processes stool, stores FMT preparations, fulfills clinicians' and researchers' requests for those preparations[139]. Taymount Clinic (UK), Chinese FMT bank, Advancing Bio (USA) and the Netherlands Donor Feces Bank (Dutch) are currently successful stool banks around the world[140].

There are many advantages of FMT application, and adverse effects of FMT were also reported. Common adverse events of FMT include abdominal discomfort, bloating, flatulence, diarrhea, constipation, vomiting, and transient fever[141, 142]. In addition, transfer of live microorganisms with undesired disease phenotypes to recipients presents a greater potential risk. High risk exists that the undesired diseases including obesity, diabetes, chronic and cardiovascular diseases and metabolic syndromes, could be transferred along with the FMT[143]. And the potential risks could be mitigated by strict selection and screening process for the donors[144]. Well-defined specific bacterial communities and their products may overcome the disadvantages of FMT and succeed FMT ultimately[145].

1.5.3 Phage therapy

Phages, short for bacteriophages, are bacteria-specific viruses that inhabit in the human intestine sharing the same ecosystem with gut microbes[146, 147]. Phages are rich in variety and quantity, accounting for about 90% of the human virome, and have a great impact on the gut microbiota. Phage therapy is widely applied as an alternative strategy to take place of antibiotics to treat bacterial infection. Phages have the potential to modulate the gut microbiota composition and reduce the number of pathogenic bacteria[148]. For the moment there are still no licensed phage therapy products for human use. The first US clinical trial of intravenously administered phage therapy is approved by American food and drug administration (FDA) in 2019[149]. And there are a few commercial phage preparations used for biocontrol of bacterial pathogens in the food industry approved by the FDA. These commercial phage preparations are specifically against *Salmonella*, *Listeria monocytogenes*, *E.*

coli O157:H7, *Mycobacterium tuberculosis*, *Campylobacter*, and *Pseudomonas syringae*[150-153]. Development and improvement in the gene editing tool CRISPR/Cas have created better opportunities for phage therapy. Engineered phage could be designed to bind specifically to the target-bacteria to shape the intestinal community to exert therapeutic effects.

1.6 OUTLINE OF THE THESIS

Pemba Island is located on the eastern coast of Tanzania and is one part of the Zanzibar Islands. The climate on the island is mild, and the temperature is kept between 24°C and 28°C throughout the year. Therefore, the residents on the island plant plenty bananas and feed on them. In addition, the residents of the island consume large amounts of vegetables and fruits, mainly cassava, rice and corn as their staple food, and fish as the main source of animal food. The parasitic diseases in Africa are highly prevalent, which seriously endangers the quality of life of the African people. The prevalence of soil-borne helminthiasis on Pemba Island is relatively high. The research center Public Health Laboratory Ivo de Carneri (PHL-IdC) hosts researchers from many countries on Pemba Island to carry out scientific research. The field part of the investigation reported in this thesis was conducted with the support of the PHL-IdC.

At present, there are very few studies on the dietary structure of African island residents. Most of the research are carried out on the dietary structure of Western countries, such as the Mediterranean diet and the typical Western diet. In this study, a total of 32 pairs (64) of mother and child volunteers on Pemba Island with similar diets were recruited in three different villages on the Island. First, we used the Food Frequency Questionnaire (FFQ) to analyze the dietary structure and food composition of women and their children. At the same time, we used 16S rDNA high-throughput sequencing technology to study the intestinal flora of the volunteers. Heat maps were utilized to show the correlation between gut microbiota and diet structure, gut microbiota and nutrient intake. We found that Pemba women and children's diets were characterized by high carbohydrate and high dietary fiber intake, low protein and low

fat intake. Supply ratios of carbohydrate, fat and protein were out of balance; The percentage of carbohydrates supply was too high, the percentage of fat supply was too low. There was a significant positive correlation between the abundance of *Faecalibacterium* and the intake of Cu, and a negative correlation between *Faecalibacterium* and the intake of fat in the intestine of healthy Pemba women. There was a significant positive correlation between *Bacteroides* and Se, and a significant negative correlation between *Bacteroides* and Cu in the intestine of healthy Pemba children (Chapter 2).

Secondly, we used the gut microbiota of healthy Spanish women and Australian children as reference to analyze the similarities and differences in the structure and function of the core intestinal microbiota of healthy women and children from Pemba Island. We found that Firmicutes and Bacteroidetes were the dominant phyla in the gut microbiota of Pemba women and children, moreover, *Prevotella* was the dominant genus. The structure of the gut microbiota in healthy Pemba women was significantly different from that of Spanish women, and the structure of the gut microbiota of Pemba children was also significantly different from the Australian children; PICRUST prediction analysis revealed that the metabolic pathways "Lipid metabolism" and "Nucleotide metabolism" showed significantly higher abundance in women and children from Pemba Island, compared with Spanish women and Australian children respectively (Chapter 3).

Then, based on the results of the Mini-Flotac parasitological examination, we divided all volunteers of Pemba Island into four groups, healthy women group, whipworm-infected women group, healthy children group, whipworm-infected children group, and used bioinformatics technology to analyze the association between the intestinal microbiota and whipworm infection. We found that, compared with healthy women and children, the diversity of the gut microbiota of whipworm-infected women and children were higher, especially in the microbiota of the infected women, the alpha diversity and beta diversity of infected women were significantly increased. Short-chain fatty acid-producing bacteria such as *Blautia* and *Bacteroides* showed

decreased level in whipworm-infected children, and short-chain fatty acid-producing bacteria such as *Prevotella* 2, *Prevotella* 9 and Ruminococcaceae UCG-005 showed decreased level in whipworm-infected women volunteers. Opportunistic pathogens such as *Enterococcus* showed increased level in whipworm-infected children, and *Campylobacter* showed elevated level in whipworm-infected women; the abundance of *Bifidobacterium* exerted decreased amount in the gut of healthy women and children with respect to the infected counterpart. Notably, in the healthy women's gut, the abundance of *Bifidobacterium* presented a significantly reduced level (Chapter 4).

Finally, we speculated that *Bifidobacterium* may have the potential to regulate IBD. Therefore, in the last chapter, we used *Bifidobacterium breve* to intervene in the mouse IBD model to explore the potential immune regulation of *B. breve*. We found that low concentration of *B. breve* could reduce the DAI index of DSS-induced mouse colitis, reduce colon shortening and slice pathological damage, and exert preventive roles. *B. breve* could promote the secretion of anti-inflammatory cytokines and inhibit the expression of pro-inflammatory cytokines in serum, at the same time, it could increase the number of $CD4^+CD25^+Foxp3^+$ Treg cells, and inhibit the number of $CD3^+CD4^+IL\text{-}17^+$T cells in mesenteric lymph nodes, to reduce intestinal injury. Low-concentration *B. breve* intervention could also improve the composition of the gut microbiota, reduce the loss of short-chain fatty acid-producing bacteria, reduce the production of pro-inflammatory bacteria, reshape the intestinal microecology and reduce intestinal inflammation (Chapter 5).

CHAPTER II ANALYSIS OF DIETARY FOOD AND NUTRITION STRUCTURE OF WOMEN AND CHILDREN IN PEMBA ISLAND

2.1 INTRODUCTION

Pemba Island is located near the east coast of Tanzania and is part of the Zanzibar archipelago. Pemba, in Arabic, translated as “green island”, is a small island with a great biodiversity and mild climate, the average temperature between 24°C and 28°C all year round. The local tourism industry is not yet developed as in the other island of the Archipelago, therefore, the resident population plants bananas that uses in a daily-based diet. In addition, local residents mainly consume cassava, rice, and corn, as staple food, and fish as the main source of animal protein.

The outbreak of parasitic diseases in Africa is common, which affects the quality of life and in some cases seriously endangers lives of African people. Soil-transmitted nematodes are highly prevalent among the residents of Pemba Island. The endemic condition of this infection and the high prevalence provides a perfect opportunity for researchers to study the parasite diseases. The research center Public Health Laboratory Ivo de Carneri (PHL-IdC), established in Pemba from 1997 by the Government of Zanzibar with funds from the Ivo de Carneri Foundation (IdCF), following the recommendations by the Italian parasitologist Ivo de Carneri and named in his memory, hosts researchers from many countries to carry out scientific research on parasitic diseases. The field part of the investigation reported in this thesis was conducted with the support of the PHL-IdC.

At the moment, there are very few studies on the dietary structure of African island inhabitants, and most of the literature focus on the diets of Western countries, such as the Mediterranean diet, typical Western diets, etc. We used the dietary frequency questionnaire (FFQ) to study the daily-based nutrition and food composition of women and their children in Pemba Island, to evaluate the nutritional characteristics of mothers

and children in Pemba Island. In addition, we also studied the structure of gut microbiota by 16S rDNA sequencing technology and displayed the correlation analysis between microbiota and diet, as well as microbiota and nutrient intake by correlation interaction maps.

2.2 MATERIALS AND METHODS

2.2.1 Recruitment of study participants

The project was approved by the Zanzibar Medical Ethical Research Committee (protocol number: ZAMREC/001/SEPT/018). Written informed consent was obtained from all subjects enrolled in the study. Women aged 23–45 years and their children aged 1.5–2.6 years from Vitongoji village, Gombani village and Chake-Chake town were recruited for this study. Thirty-two mothers-and-children pairs of volunteers were enrolled. During the visits of the mothers to the sanitary center for their children's routine examinations, the mothers were interviewed. The questionnaire was proposed to each volunteer. To facilitate reciprocal understanding, interviews were performed in Swahili with the help of nurses and personnel of the Public Health Laboratory Ivo de Carneri (PHL-IdC). The inclusion criteria for all the individuals were as follows: similar nutritional habits (similar origin of food and diet, mainly consisting of banana fruit, cassava, rice, cassava leaves as vegetable and dagaa fish), no pregnancy, no HIV, no diarrhea, no fever, no diabetes, no malaria, and no antibiotics or anthelmintic treatment in the previous 3 months. The questionnaire format is included in the appendix.

2.2.2 Analysis of food structure and nutrients.

Based on the questionnaire collected from the volunteers on Pemba Island, dietary structure and nutrients intake were analyzed. Since some foods are traditional and local specialties, so we took the dietary guidelines released from Tanzania Food and Nutrition Centre as a reference (https://www.hsph.harvard.edu/nutritionsource/food-tables/). These reference tables provide the amounts of amino acids, macronutrients, minerals, vitamins referred to the typical Tanzanian food. In addition, we compared the nutrients intake results with the reference intakes of dietary

nutrients for Chinese residents to evaluate the nutrimental status of Pemba volunteers. Estimated average requirement (EAR) and recommended nutrient intake (RNI) are the two evaluation indicators of nutrient intake that we utilized in the study. EAR is the value of a nutrient that is estimated to meet the requirement for a specific criterion of adequacy of half of the healthy individuals of a specific age, sex, and life-stage[154]. RNI is the daily intake, which meets the nutrient requirements of almost all (97.5 percent) apparently healthy individuals in a specific age and sex group[155].

2.2.3 Fecal sample collection and parasitological analysis

All the participants were provided with sterile containers to collect fecal samples. Each sample was well homogenized and divided into three aliquots. One aliquot was used for parasitological examination directly, one for the DNA extraction, performed upon sample reception, and the third was maintained frozen as a backup. The Mini–FLOTAC technique was utilized for microscopic examination[156] . Two grams of stool sample and 2 ml of 5% formalin were mixed into the conical collector first. After the samples were homogenized sufficiently, flotation solution (saturated sodium chloride) was added to a volume of 40 ml. After another round of homogenization, the samples were added to the two flotation chambers. Finally, after waiting for 10 min, the numbers of eggs per gram of feces were determined under a microscope. Analytic sensitivity could reach ten eggs per gram of feces[157]. This analysis was repeated twice for each sample. All procedures were conducted in the laboratory of the PHLIdC. After the parasitological analysis was performed, the results were delivered to the enrolled women participants to ensure that they could go to the sanitary center to receive anti-helminth treatment. The treatment was given immediately by the PHL team since they also have this program among their activities. Infected participants were treated with albendazole drugs.

2.2.4 DNA extraction, PCR amplification, 16S rRNA gene sequencing

Total DNA was extracted from fecal samples utilizing the PureLink™ Microbiome DNA Purification Kit (Invitrogen, Waltham, Massachusetts, USA) according to the manufacturer's instructions. The integrity and concentrations of the obtained DNA were

determined by agarose gel electrophoresis and NanoDrop spectrophotometer, respectively. Then, the V3-V4 hypervariable region of the 16S rRNA gene was amplified by PCR. The universal primers (forward, Pro341: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG-3′; reverse, Pro805: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC-3′) were used [158]. PCR was performed in triplicate in 25 μL volumes containing 2.5 μL of 10× Pyrobest Buffer, 2 μL of 2.5 mM dNTPs, 1 μL of each primer (10 μM), 0.4 U of Pyrobest DNA Polymerase (TaKaRa), and 15 ng of template DNA. The PCR program involved an initial denaturation step at 94 ℃ for 3 min followed by 25 cycles of denaturation at 94 ℃ for 30 s, annealing at 50 ℃ for 30 s and extension at 72 ℃ for 60 s with a final extension phase at 72 ℃ for 7 min.

PCR products were run in an electrophoresis chamber on a 1% agarose gel, and 50 ng of purified DNA extraction of each sample was subsequently prepared and sent to the BMR Genomics company (Padova, Italy) for sequencing. Sequencing libraries were generated using the NEBNext® Ultra™ DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendations. Library quality was assessed and sequenced on an Illumina MiSeq platform PE300 platform (Illumina, San Diego, CA, USA).

2.2.5 Bioinformatics and Sequencing Data Analysis

The original DNA fragments were trimmed of the adapters using Cutadapt (version 1.18; https://cutadapt.readthedocs.io/en/stable/index.html) [159]. FastQC was applied to check the quality of the raw reads after the trimming process (version 0.11.8; http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The raw reads were processed utilizing QIIME2 (Quantitative Insights Into Microbial Ecology 2, version 2018.11; https://qiime2.org) [160]. Joined sequences were successfully quality-filtered and dereplicated with identification of chimeras. Paired-end reads were merged in QIIME2 using the DADA2 pipeline (version 1.10; https://benjjneb.github.io/dada2/index.html). Sequences were clustered into

operational taxonomic units (OTUs) according to the taxonomy assignment through annotation against the SILVA database (version SSU138; https://www.arb-silva.de). Sequences that did not match references in the SILVA database were clustered de novo on the basis of pairwise sequence identity (cut-off: 99% sequence similarity). The first selected cluster seed was considered the representative sequence of each OTU. The OTU table with the assigned taxonomy was exported from QIIME2 alongside an unweighted UniFrac distance matrix. Prior to downstream analyses, we removed singleton OTUs. Cumulative-sum scaling (CSS) was applied followed by log2 transformation to account for the non-normal distribution of taxonomic count data. QIIME2 evaluated three metrics of alpha diversity, including observed OTUs, estimating the microbial richness; Shannon index, estimating species biodiversity; and Faith's phylogenetic diversity index, measuring community richness incorporating phylogenetic relationships between the features. Differences in alpha diversity were tested by the Wilcoxon rank-sum test. Principal component analysis (PCA) of the OTUs in different groups was performed based on the unweighted UniFrac distance values and statistical differences were calculated by Permutation Multivariate Analysis of Variance (PERMANOVA) function with 999 permutations. Furthermore, a linear discriminant analysis (LDA) effect size (LEfSe) algorithm (http://huttenhower.sph.harvard.edu/galaxy/) was performed to identify the significant microbial differences among all the groups [161]. This analysis, based on the nonparametric factorial Kruskal-Wallis and on the (unpaired) Wilcoxon rank-sum test, was also validated with a further Student's t-test using the STAMP software (version 2.1.3). Microbial community metagenome functions were predicted by Qiime2 PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, version 2019.7; https://github.com/picrust/picrust2/) [162]. PICRUSt2 comparisons between each group were performed using STAMP with Student's t-test (version 2.1.3). P-values < 0.05 were considered to be significant. Spearman correlation heatmap was conducted using R Script ggplot2 (https://ggplot2.tidyverse.org/).

2.2.6 Sequence read accession number

The raw reads were deposited into the NCBI Sequence Read Archive database (SRA accession number: SRP259507, BioProject accession number: PRJNA629760).

2.3 RESULTS

2.3.1 Characteristics of the participants

32 pairs of mothers and children were interviewed, provided their consent and the fecal samples. 20 (12 mothers and 8 children) samples were positive for helminth infection and 44 (20 mothers and 24 children) samples were negative for helminth infection. 12 infected mother individuals consisted of 9 *T. trichiura*-infected individuals, 1 *A. lumbricoides*-infected one and 2 individulas with combined infection of *T. trichiura* and *A. lumbricoides*. 8 infected children concluded 5 *T. trichiura*-infected ones, 2 *A. lumbricoides*-infected ones and one individula with combined infection of *T. trichiura* and *A. lumbricoides*. The infection status, demographics of the mothers and children are shown in Table 1. Sample codes of all the 32 pairs of mothers and children and infection burdens of helminth-infected participants are shown in Table 2-1.

Tab.2-1 Helminth infection and worm burden of mothers and children on Pemba Island

Sample code of mothers	Villages	T. trichiura （EPG）	A. lumbricoides （EPG）	Corresponding sample code of children	T. trichiura （EPG）	A. lumbricoides （EPG）
M01	Vitongoji	10	10	C31	0	0
M02	Vitongoji	0	0	C32	20	0
M04	Vitongoji	200	200	C34	0	10
M05	Vitongoji	20	0	C35	100	0
M06	Vitongoji	20	0	C36	0	0
M07	Vitongoji	0	0	C37	0	0
M08	Vitongoji	0	0	C38	0	0
M09	Vitongoji	450	0	C39	0	0
M10	Vitongoji	0	0	C40	0	0
M11	Vitongoji	20	0	C41	30	0
M12	Gombani	0	0	C42	0	0
M13	Gombani	0	0	C43	0	0
M14	Gombani	0	0	C44	0	0

M16	Gombani	0	0	C46	0	4000
M17	Gombani	160	0	C47	80	0
M18	Gombani	100	0	C48	0	0
M19	Gombani	0	0	C49	0	0
M20	Gombani	0	0	C50	0	0
M22	Gombani	0	0	C52	0	0
M23	Gombani	700	0	C53	250	750
M25	ChakeChake	0	0	C55	0	0
M26	Chake	0	0	C56	0	0
	Chake					
M28	Chake	0		C58	0	0
	Chake					
M30	Chake	150	0	C60	0	0
	Chake					
M61	Chake	70	0	C81	0	0
	Chake					
M62	Chake	0	0	C82	0	0
	Chake					
M63	Chake	0	0	C83	0	0
	Chake					
M64	Chake	0	0	C84	0	0
	Chake					
M65	Chake	0	0	C85	0	0
	Chake					
M66	Chake	0	2000	C86	0	0
	Chake					
M67	Chake	0	0	C87	70	0
	Chake					
M69	Chake	0	0	C89	0	0
	Chake					

2.3.2 Analysis of the diet of mothers and children in Pemba Island

The detailed physical information of all the volunteers were recorded. The diet structure of the volunteers were shown in Table 2-2. The age, height, body weight and body mass index of the mother volunteers were 30.47 ± 5.842 years, 157.8 ± 6.244 cm, 55.64 ± 7.513 kg, 22.32 ± 2.513 kg/m^2, respectively. For the children volunters, the age,

height, body weight and body mass index were 1.791 ± 0.3514 years, 79.28 ± 4.343 cm, 10.55 ± 1.407 kg, 16.78 ± 1.776 kg/m2, respectively. Preliminary analysis of the diet showed that mothers and childlren were both characterized by a high intake of cereals and staple foods (as source of carbohydrates), a high intake of vegetables and fruits (as source of fiber), and a small intake of animal-derived foods.

Tab.2-2. Structure of the diet of mothers and children individuals in Pemba Island (grams/day)

Sample ID	ugali	rice	cassava	makubi	banana	fish	red meat	white meat	vegetables	beans	chapati	dagaa	potatoes	mandazi	bread	fruits	tea	egg
M01	300	150	200	200	300	150	0	0	100	30	50	50	0	30	0	100	20	0
M02	350	150	180	200	150	100	0	0	80	15	50	0	0	0	0	75	0	0
M04	250	100	250	150	300	100	0	0	150	15	0	0	0	0	0	50	20	0
M05	350	150	200	300	150	100	0	0	100	75	100	150	0	30	0	50	0	0
M06	300	100	240	200	150	80	50	0	100	50	50	50	0	60	0	65	20	50
M07	350	130	220	100	300	150	0	50	100	35	100	0	0	0	0	100	0	0
M08	300	200	200	100	150	100	0	0	200	50	50	100	150	60	0	50	20	0
M09	250	150	250	150	150	120	0	0	150	50	50	50	0	0	0	75	20	0
M10	300	200	150	200	300	100	0	0	120	40	50	0	0	30	0	50	20	0
M11	350	150	250	150	150	100	0	0	100	35	50	50	0	0	0	100	40	0
M12	200	250	100	100	150	150	0	0	200	70	0	50	0	0	0	100	20	0
M13	250	250	250	100	150	200	0	0	125	65	50	0	0	30	0	50	0	0
M14	300	100	150	250	150	100	0	50	200	100	50	0	0	0	0	50	20	0
M16	200	300	200	100	150	100	0	0	150	100	100	100	0	30	0	65	40	0
M17	250	300	150	150	150	100	0	0	100	50	0	100	0	30	0	75	20	0
M18	200	400	150	100	300	100	0	0	120	65	50	0	0	0	0	55	0	0
M19	250	150	200	200	150	150	0	0	150	65	50	50	0	0	0	50	20	0
M20	300	200	180	150	300	120	0	0	100	80	0	0	0	0	0	35	20	0
M22	300	100	250	100	150	100	0	0	95	55	50	100	0	30	0	60	40	0
M23	350	100	150	300	150	85	50	0	135	60	100	100	0	60	100	50	20	0
M25	200	200	175	150	300	90	0	0	80	80	0	100	0	30	0	60	40	0
M26	150	300	250	200	150	100	0	0	150	30	50	0	150	30	0	85	20	0
M28	350	100	200	150	300	110	0	0	200	50	50	0	0	0	0	60	20	50
M30	200	150	250	100	150	100	0	0	250	50	100	100	0	30	0	60	0	0
M61	250	200	200	250	300	100	0	0	100	50	50	0	0	30	50	55	20	0

M62	350	150	150	200	150	100	0	50	90	30	0	0	0	0	0	35	0	0
M63	300	150	200	200	300	80	0	0	100	25	50	50	0	0	0	45	20	0
M64	250	250	150	150	150	75	50	0	85	20	0	50	0	60	100	80	0	0
M65	300	150	150	250	150	100	0	0	200	30	100	0	100	0	0	65	0	0
M66	350	100	200	150	300	100	50	0	100	50	0	50	0	30	0	60	20	0
M67	300	200	150	200	150	100	0	0	150	100	50	50	0	0	0	45	0	0
M69	200	150	200	150	300	100	50	0	100	30	50	0	0	0	0	70	20	0
C31	100	80	50	25	25	20	0	0	25	10	0	0	0	0	0	20	0	0
C32	100	100	30	25	20	15	0	0	20	0	0	0	0	0	0	15	0	0
C34	150	50	50	25	15	10	0	0	25	10	0	0	0	0	0	25	0	0
C35	50	50	150	25	10	20	0	0	15	5	0	15	0	0	0	20	0	0
C36	80	30	100	50	15	30	0	0	30	5	0	0	0	0	0	20	0	0
C37	100	30	50	50	20	10	0	0	20	0	0	0	0	0	0	30	0	0
C38	80	100	30	25	15	50	0	0	25	5	0	0	0	0	0	25	0	0
C39	80	90	30	25	30	25	0	0	30	0	0	0	0	0	0	35	0	0
C40	100	50	30	50	20	50	0	0	50	10	0	0	0	0	0	25	0	0
C41	80	90	30	25	25	5	0	0	50	10	0	0	0	0	0	30	0	0
C42	100	50	50	25	20	15	0	0	30	5	0	0	0	0	0	25	0	0
C43	50	90	80	0	30	30	0	0	25	25	0	0	0	0	0	15	0	0
C44	90	80	50	25	50	20	0	0	30	0	0	0	0	0	0	20	0	0
C46	50	100	30	50	15	25	0	0	35	10	0	0	0	0	0	30	0	0
C47	80	50	30	0	20	10	0	0	50	15	0	0	0	0	0	25	0	0
C48	80	100	30	25	25	15	0	0	20	5	0	0	0	0	0	45	0	0
C49	100	50	50	25	30	20	0	0	45	0	0	0	0	0	0	35	0	0
C50	80	80	30	25	10	15	0	0	25	10	0	0	0	0	0	30	0	0
C52	50	100	30	50	25	30	0	0	50	0	0	0	0	0	0	35	0	0
C53	50	90	30	50	50	20	0	0	30	0	0	0	0	0	0	25	0	0
C55	80	90	30	25	20	25	0	0	50	5	0	0	0	0	0	35	0	0
C56	100	50	30	25	25	35	0	0	30	10	0	0	0	0	0	20	0	0
C58	100	50	30	50	60	15	0	0	35	5	0	0	0	0	0	40	0	0

C60	80	90	30	25	30	20	0	0	45	20	0	0	0	0	0	50	0	0
C81	80	50	30	0	80	20	0	0	30	15	0	0	0	0	0	25	0	0
C82	100	50	30	50	20	35	0	0	30	5	0	0	0	0	0	20	0	0
C83	80	30	50	50	65	45	0	0	45	0	0	0	0	0	0	30	0	0
C84	100	50	30	25	55	10	0	0	35	10	0	0	0	0	0	35	0	0
C85	100	50	30	25	35	15	0	0	55	5	0	0	0	0	0	50	0	0
C86	50	100	30	0	55	25	0	0	30	0	0	0	0	0	0	30	0	0
C87	50	100	30	0	10	20	0	0	45	5	0	0	0	0	0	45	0	0
C89	50	80	30	25	45	25	0	0	35	15	0	0	0	0	0	60	0	0

2.3.3 Analysis of nutrients intake of mothers and children in Pemba Island

Compared with the RNI from the Chinese dietary guidelines (the version published in year of 2013), more specifically, with respect to the RNI of the Chinese women of the same age period, women volunteers in Pemba island reached the standard rate of 87.5% for protein, 100% for carbohydrates, 21.88% for vitamin B1, 6.25% for zinc, 28.13% for copper, while vitamin A, vitamin C, calcium, magnesium, iron, phosphorus, potassium, selenium and manganese intake were all substandard. Moreover, Pemba women' daily intakes of vitamin A, vitamin C, calcium, magnesium, phosphorus and selenium showed a lower EAR compared to the one of Chinese women. Furthermore, in children volunteers of the island, the RNI of vitamin A, vitamin C, vitamin B1, calcium, magnesium, iron, phosphorus, zinc, copper, potassium, selenium were under the standard rate of the similar aged children in China. What's more, the EAR of protein, carbohydrates, vitamin A, vitamin C, vitamin B1, calcium, magnesium, zinc and selenium were all lower than those of the children of same age in China, as shown in Tables 2-3 and 2-4. Information on food nutrient intakes for all Pemba volunteers is provided in Appendix I.

2.3.4 Heat map of the dietary structure of mothers and children in Pemba Island

As shown in Figures 2.1 and 2.2, the ordinate in the figure represents the type of food, and the abscissa represents the volunteers in Pemba Island. The colorful axis transforms from red to yellow, green and blue, and the transformation of color corresponds to a gradual decrease in food consumption. It is reported from the heat map that the diet structure of Pemba mother and child volunteers is very similar. In their daily life, they mainly rely on ugali, rice, cassava, vegetables, bananas, and fish, and their diet is relatively simple.

Tab.2-3 Daily nutrient intakes of women on Pemba Island

Nutrients	Daily nutrients intake of mothers in Pemba Island	EAR of Chinese women of the same age period	RNI of Chinese women of the same age period	Percentage of Pemba mother reaching the RNI of the Chinese women （%）
protein/g	67.30 ± 7.88	50	55	87.5
fat/g	45.53 ± 8.97	-	-	-
carbohydrates/g	275.7 ± 47.71	120	-	100
fiber/g	26.06 ± 4.06	-	-	-
vitamin A/μg	155.8 ± 4.42	480	700	0
vitamin C/mg	51.7 ± 4.30	85	100	0
vitamin B1/mg	1.09 ± 0.13	1	1.2	21.88
calcium/mg	238.2 ± 8.28	650	800	0
magnesium/mg	246.2 ± 6.66	280	330	0
iron/mg	14.54 ±1.40	9	20	0
phosphorus/mg	478.70 ± 4.54	600	720	0
zinc/mg	7.08 ± 0.32	6	7.5	6.25
copper/mg	0.70 ± 0.14	0.6	0.8	28.13
potassium/mg	1422 ± 57.08	-	2000	0
selenium/mg	38.40 ± 1.23	50	60	0
manganese/mg	0.96 ± 0.13	-	4.5	0

Tab.2-4 Daily nutrient intake of children on Pemba Island

Nutrients	Daily nutrients intake of children in Pemba Island	EAR of Chinese children of the same age period	RNI of Chinese children of the same age period	Percentage of Pemba children reaching the RNI of Chinese children（%）
protein/g	20.16 ± 2.04	25	30	0
fat/g	14.81 ± 1.50	-	-	-
carbohydrates/g	94.02 ± 3.13	120	-	-
fiber/g	7.27 ± 1.01	-	-	-

vitamin A/μg	25.48 ± 1.90	220	310	0
vitamin C/mg	10.82 ± 1.29	35	40	0
vitamin B1/mg	0.21 ± 0.05	0.5	0.6	0
calcium/mg	59.39 ± 2.35	500	600	0
magnesium/mg	54.62 ± 3.03	110	140	0
iron/mg	4.77 ± 0.85	6	9	0
phosphorus/mg	89.39 ± 2.64	250	300	0
zinc/mg	1.05 ± 0.18	3	4	0
copper/mg	0.12 ± 0.05	0.25	0.3	0
potassium/mg	349.7 ± 5.93	-	900	0
selenium/mg	10.3 ± 1.434	20	20	0
manganese/mg	0.14 ± 0.04	-	-	-

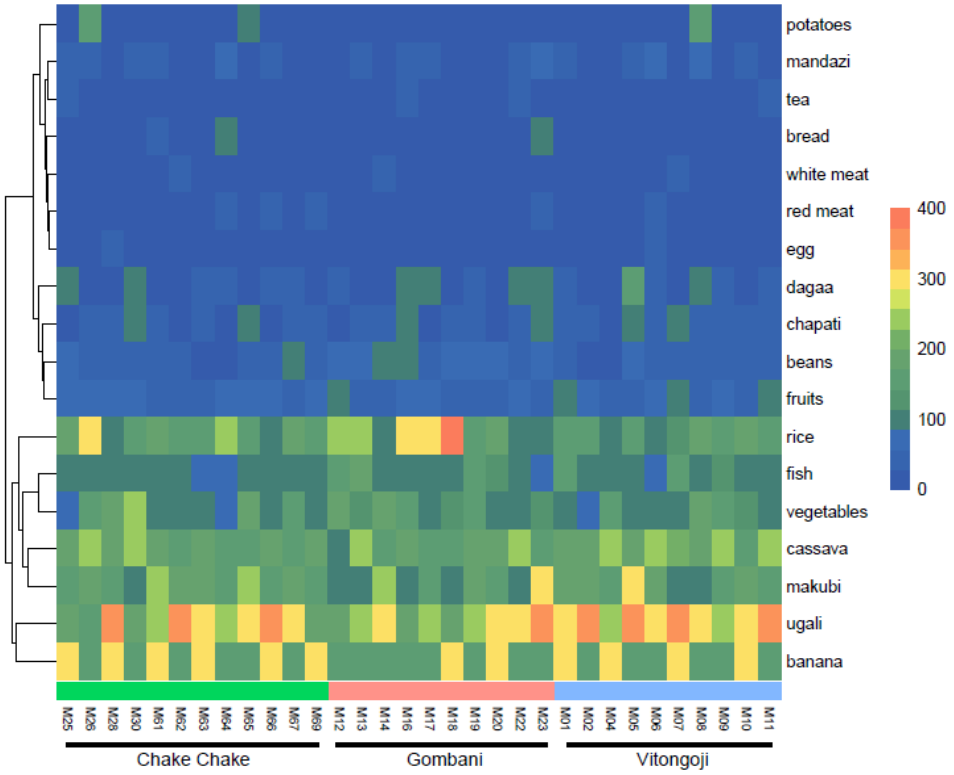

Fig.2.1 Heat map of dietary structure of women on Pemba Island

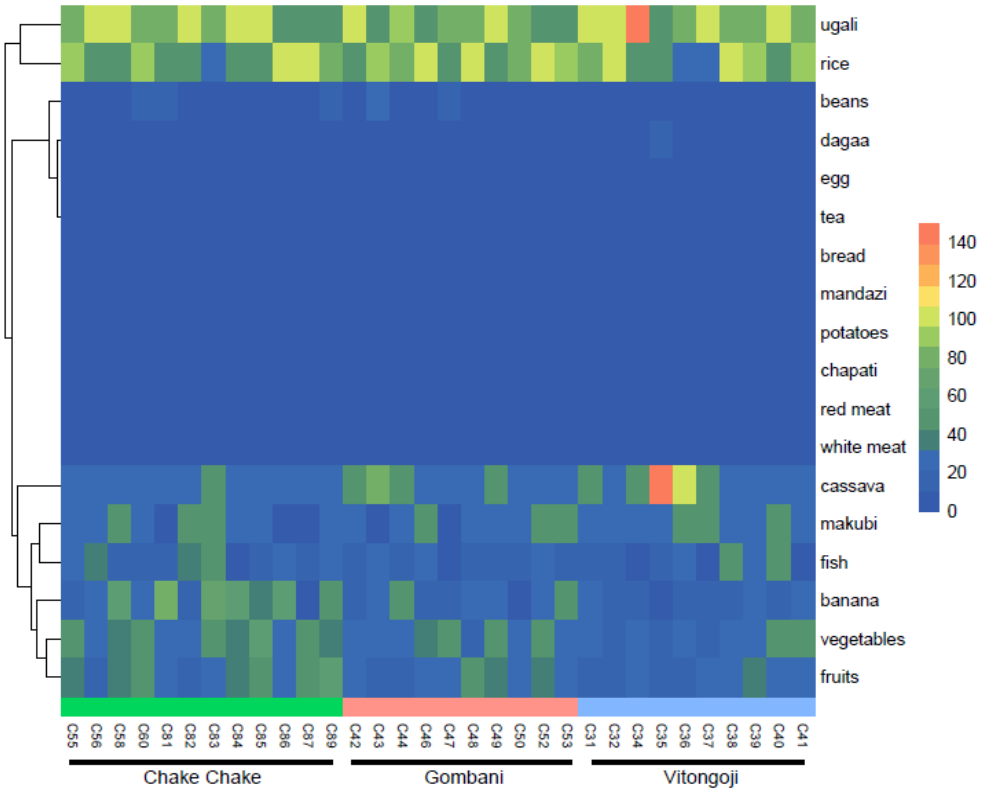

Fig.2.2 Heat map of dietary structure of children on Pemba Island

2.3.5 Correlation analysis of gut microbiota and diet in health individuals in Pemba island

The Mini-Flotac test's results showed that 20 stool samples were positive for helminth infection. The other 44 stool samples were neither positive for helminth infection nor for protozoan infecction, although it is more difficult to detect protozoa infection by Mini-Flotac. According to the parasite infection status, we divided the individuals into 44 healthy ones and 20 helminth-infected ones. Based on the 44 healthy volunteers, we performed the correlation analysis of their gut microbiota and diet. However, due to the poor quality of the fecal samples or DNA extraction problems, the raw sequencing data from 5 healthy volunteers (sample numbers M20, M62, C44, C83 and C85) were invalid and discarded for the further 16s sequencing. Eventually, we conducted the analysis among the remaining 39 healthy volunteers. As shown in Figures 2.3 and 2.4, heat maps of the diet associated with the corresponding gut flora

of 18 women and 21 children were constructed respectively. The heat map in Figure 2.3 displays the direct correlation between the frequency of consuption of various foods in the diet and the relative abundance of gut microbiota. The abscissa of the heat map represents the amount of food intake, and the ordinate represents the abundance of gut microbiota at the genus level. The color changes from red, through yellow to blue, represent the transition from positive correlation to negative correlation between food intake and the abundance of intestinal flora. The results showed that there is a significant positive correlation between *Bifidobacterium* and legumes, *Bifidobacterium* and dagaa in healthy women's diet, indicating that the women with higher intake of legumes or dagaa were usually associated with higher levels of *Bifidobacterium* in the gut. *Faecalibacterium* and makubi, *Roseburia* and makubi, all had a significant positive correlation, *Ruminobacter* had a significantly positive correlation with banana. *Lachnospira* had a significantly negative correlation with ugali. Moreover, *Subdoligranulum* had a significantly negative correlation with ugali. As for the dietary structure of children volunteers, *Bacteroides* and fish, *Parabacteroides* and rice, *Faecalibacterium* and banana, *Alloprevotella* and makubi, all had significantly positive correlations respectively. *Succinivibrio* also had a very significantly positive correlation with makubi .

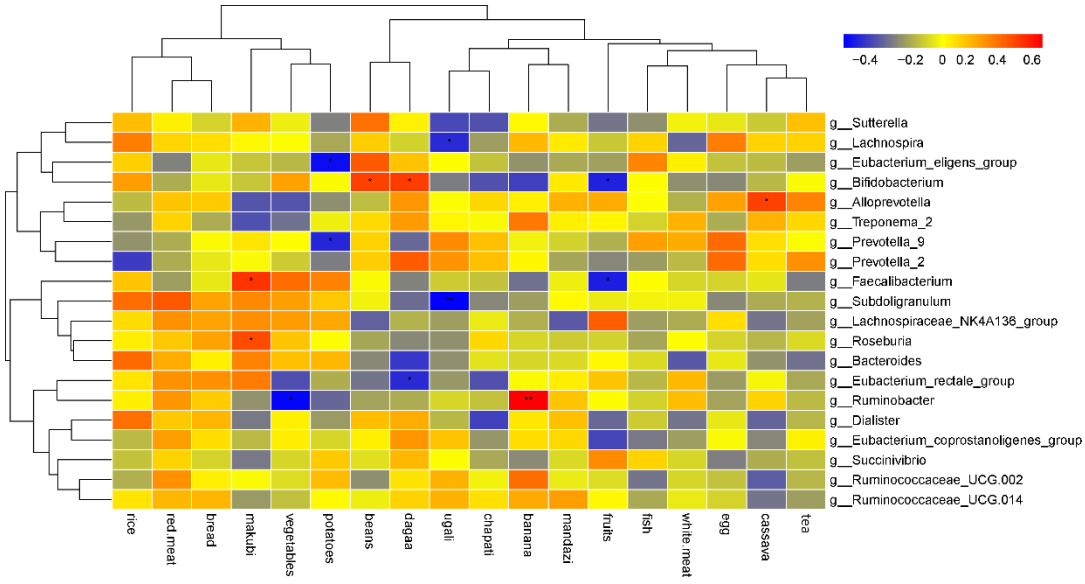

Fig.2.3 Correlation heat map of gut microbiota and dietary structure in healthy women

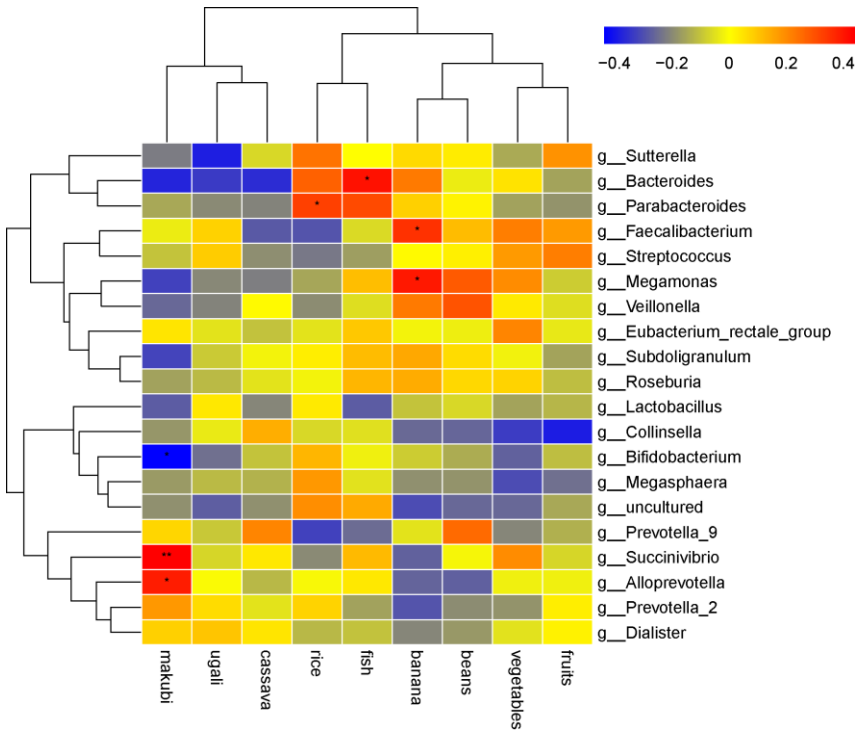

Fig.2.4 Correlation heat map of gut microbiota and dietary structure in healthy children

2.3.6 Correlation analysis of gut microbiota and nutrients in healthy individuals in Pemba island

The correlation between the intake of various nutrients and the abundance of the gut microbiota can be described directly by the heat map. The abscissa of the heat map represents the nutrients intake, and the ordinate represents the abundance of gut microbiota at the genus level. The color changes from red, through yellow to blue, representing the transition from positive correlation to negative correlation between nutrients intake and the abundance of intestinal microbes. As shown in Figures 2.5 and 2.6, there was a significant positive correlation between *Faecalibacterium* and Cu, a negative correlation between *Faecalibacterium* and fat in the diet structure of Pemba healthy women, indicating that the diet structure with a higher intake of Cu was usually associated with higher levels of *Faecalibacterium*, while diet structure with intake of more fat was associated with lower numbers of *Faecalibacterium* in women. In addition,

there were significant positive correlations between *Sutterella* and Ca, *Sutterella* and Cu, *Dialister* and K, respectively. The Lachnospiraceae NK4A136 group was associated with the administration of vitamin A, and associated with the administration of vitamin C negatively. In addition, *Alloprevotella* was associated positively with the administration of vitamin C and associated with Cu negatively. Moreover, there were significant negative correlations between *Lachnospira* and Zn, Ruminococcaceae UCG-002 and Ca, Ruminococcaceae UCG-014 and Ca, respectively. As for the healthy children individuals, there were significant positive correlations between *Bacteroidetes* and Se, *Parabacteroides* and protein, *Parabacteroides* and carbohydrate, *Veillonella* and Ca, separately. Besides, there were significant nagetive correlations between *Bacteroidetes* and Cu, *Parabacteroides* and Cu, *Faecalibacterium* and P, *Subdoligranulum* and P.

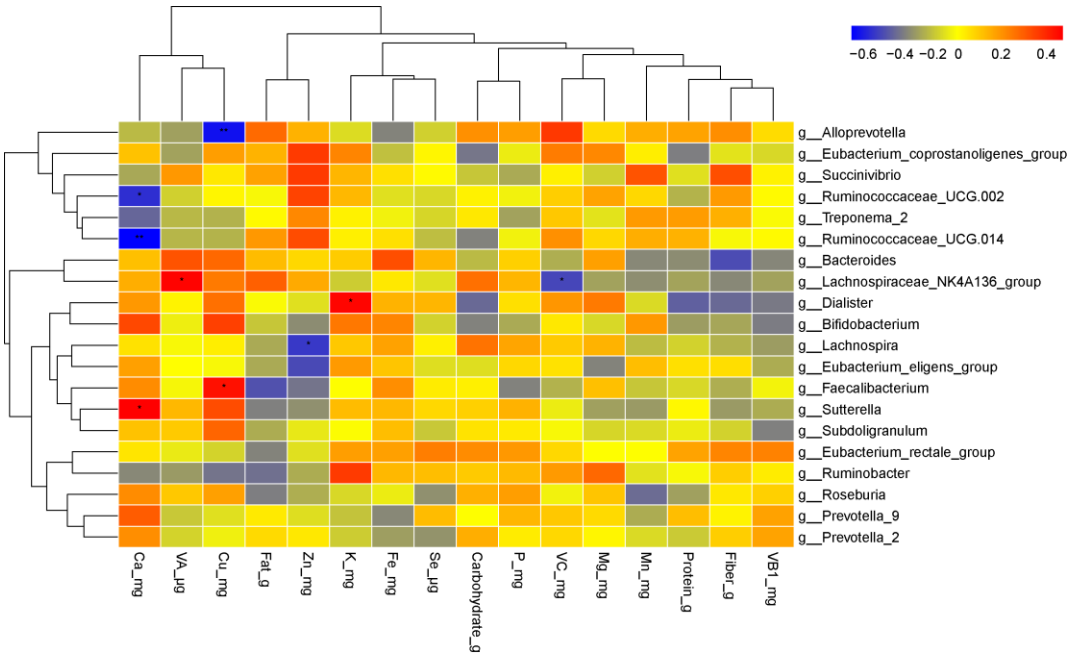

Fig.2.5 Correlation heat map of gut microbiota and dietary nutrients in healthy women

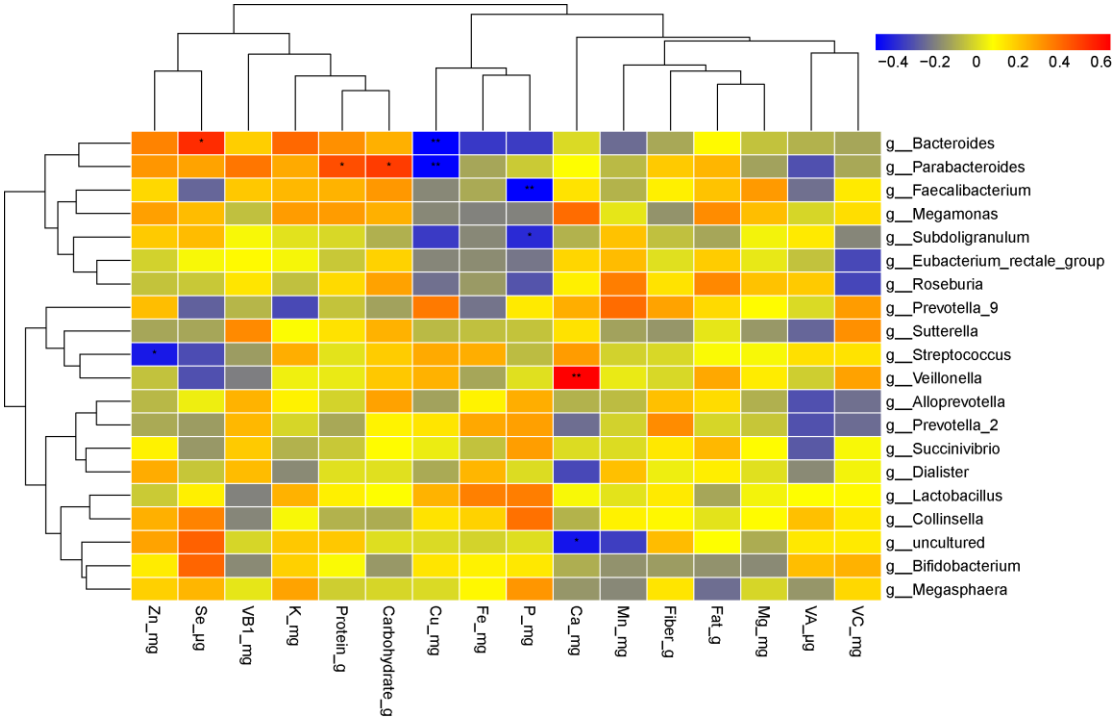

Fig.2.6 Correlation heat map of gut microbiota and dietary nutrients in healthy children

2.4 DISCUSSION

So far, there were very few research focusing on the dietary structure and intestinal microbiota of African urban residents, African rural farmers and herdsmen, and African prairie hunters [163-165]. And to the best knowledge of us, there is no literature reporting the dietary structure of African island residents. This study was the first that investigates the dietary structure of 32 pairs of mothers-children volunteers in Pemba Island, Tanzania. 16s high-throughput sequencing technology was used to analyze the composition of the microbiota. Bioinformatic tools were utilized to reveal the correlation between typical dietary structure and gut microbiota in the volunteers.

By analyzing the diet and nutritional structure of 32 pairs of mother-child

volunteers, it could be inferred that the characteristics of all the volunteers were high dietary fiber intake and low fat intake, which was consistent with the fact that the diet structure of Pemba volunters is mainly based on plant-derived foods, with less consuption of animal-derived foods. Compared with the RNI and EAR parameters of Chinese women and children with a similar age like Pemba mother and children volunteers, the EAR of protein and carbohydrate in Pemba women were significantly higher than those in Chinese women, while the EAR of protein and carbohydrate in Pemba children were lower than those in Chinese children. The RNI of vitamin A, vitamin C, vitamin B1, Ca, Mg, Fe, P, and Se in Pemba women were all lower than the average daily recommended nutrient intakes of women in China[156]. In addtion, The RNI of vitamin A, vitamin C, vitamin B1, Ca, Mg, Fe, P, Zn, Cu and Se in Pemba children were all lower than those of children in China. Life in Pemba island is far from the Chinese standard and the socio-economical context is one of the main cause of undernutrition. Moreover, the proportion of three major nutrients in the volunteers on Pemba Island was unbalanced. Carbohydrates are higher than the maximum recommended value (50%-60%). The proportion of fat is lower than the recommended value (20%-30%). Given that the dietary structure of mother-child volunteers was quite simple, plant-derived food rich in fiber and carbohydrates was the major food source, and fish was almost the only source for protein and fat. This kind of diet was unbalanced and it was similar to the dietary structure of rural residents in China in the early 20th century[166]. With the improvement of the material conditions of the inhabitants on Pemba island, the consumption of livestock or poultry meat may increase with time, then the nutrition status of the inhabitants might be improved.

The diet of Pemba volunteers shows a few similarity to the Mediterranean food diet, considering that is highly based on vegetables, with large use of fish as source of animal derived food. Distinctive from Mediterranean food diet, the intake of dairy products like cheese, nuts, olive oil,or red wine is deficient[167]. Peng et al. reported the dietary features of the indigenous ethnic Li minority people living on Hainan island and analyzed their core gut microbiota. They found that ethnic Li's diet is relatively simple,

mainly concentrating on plant-dervied food like gourd, radish and cabbage, animal-dervied food like fish and sea snail, fruits like coconuts, mangoes and bananas which are large-scale planted on the island. Ethnic Li's diet is very similar to the diet of Pemba residents[168]. Moreover, there was also a significant positive correlation between *Faecalibacterium* and Cu, a negative correlation between *Faecalibacterium* and fat in the diet structure of ethnic Li.

Our results showed that ugali, cassava, rice, vegetables, fruits and fish highly present in the diet of the Pemba volunteers characterized their diet with a high intake of fiber and polysaccharide. Ugali and cassava are the most important staple food. The main component of ugali is corn, which is a source of linoleic acid, sitosterol and vitamins, and also has biological activities such as antioxidant, anti-tumor, hypoglycemic, and immunity boostor[169-171]. Cassava is rich in nutrients such as starch, cellulose, and vitamins, and has beneficial effects on preventing hypertension or diabetes, protecting liver, and anti-oxidation[172, 173]. Yatsunenko et al. reported that the diet of African Malawis and American Indians, and found that both Malawi and Indians rely on maize and cassava as staple foods, and they had lower risk of suffering chronic diseases and cancer[174, 175]. Dietary fiber and polysaccharide could regulate intestinal epithelial cell receptors such as GLP-2, thereby enhancing the integrity of the intestinal wall, improving intestinal function, and reducing the content of pro-inflammatory cytokines such as IL-6 and the inflammatory marker MCP-1 , promoting the gut health of the host[176, 177]. Pemba residents have healthy dietary patterns, however, the local hygiene conditions are still very poor. They eat hand-picked rice and hand-picked vegetables. The hygienic are precarious, which is responsible for the spread of some parasite, bacteria and viral diseases.

2.5 CONCLUSIONS

The diet of mother and children volunteers on Pemba Island is characterized by high carbohydrate and fiber intake, low protein and fat intake.

The macronutrients are unbalanced in the diet of mother and children, indeed the

proportion of carbohydrates is too high, and proportionof fat is too low.

There was a significant positive correlation between *Faecalibacterium* and Cu, a negative correlation between *Faecalibacterium* and fat in the diet structure of women.

There were significant positive correlations between *Bacteroides* and Se, *Parabacteroides* and proteins, *Parabacteroides* and carbohydrates, respectively. And there were significant negative correlations between *Bacteroides* and Cu, *Parabacteroides* and Cu.

CHAPTER III CORE GUT MICROBIOTA OF HEALTHY WOMEN AND CHILDREN IN PEMBA ISLAND

3.1 INTRODUCTION

The Western diet is characterized by high sugar, high fat and high protein intake. The enterotype of the individuals that follow the Western food diet is dominated by *Bacteroides*. The enterotype of the African individuals is dominated by *Prevotella*, whose diet structure is characterized by high fiber and high carbohydrate intake. At present, under the period of big data, there is an increasing number of research based on the next generation sequencing of gut microbiota in western urban residents, while there are still very few reports on the sequencing results of African individuals lining in towns or villages.

There are several studies that have compared the gut microbiota of urban populations with agricultural populations or traditional hunter-gatherer. The individuals from agricultural societies or hunter tribe usually consume foods that are low in animal-derived protein and fats, and high in fiber and carbohydrates[22, 25, 178]. The urban-industrialized populations have higher abundance of *Bacteroides* in the gut, while the individuals from agricultural societies or hunter tribe often have higher abundance of *Prevotella* in the gut[175, 179-183]. Different types of food resources, cultural or social discrepancy, geographic and genetic factors all are involved in the difference in the gut microbiota. Although there are very few studies of microbiome in African populations[165, 175, 180, 184, 185], core gut microbiota of inhabitants on Pemba Island has not been reported yet.

We selected and downloaded raw data of gut microbiota in 21 healthy Spanish women and 37 healthy Australian children from NCBI Sequence Read Archive and European Nucleotide Archive, then by combining with the data of Pemba mother-child volunteers, we performed further bioinformatics analysis in order to understand similarities and differences in the structure and function of the core gut microbiota of mother and child in Pemba Island.

3.2 MATERIALS AND METHODS

3.2.1 Participants

According to the results of Chapter II, there are 39 healthy volunteers including 18 women and 21 children from Pemba Island, Tanzania. In this Chapter, we selected 21 healthy Spanish women and 37 healthy Australian children as control groups, to explore the differences in the structure and function of the gut microbiota between a typical African diet and a Western diet. The results are generally reported four groups: Pemba women group (M), Pemba children group (C), Spanish women group (SW), Australian children group (AC).

3.2.2 DNA extraction, PCR amplification, 16S rRNA gene sequencing

Please see 2.2.4 in chapter II.

3.2.3 Bioinformatics and Sequencing Data Analysis

Please see 2.2.4 in chapter II.

3.2.4 Sequence read accession number

The raw reads of Spanish women were deposited in the NCBI Sequence Read Archive database, BioProject accession number: PRJNA350839. The raw reads of Australian children were deposited in European Nucleotide Archive, BioProject accession number: PRJEB14969.

3.3 RESULTS

3.3.1 Analysis of macronutrients intake in women and children from Pemba Island

Protein, fat and carbohydrates serve as macronutrients to provide energy in the human body, and the ratio of these three is usually used to analyze the completeness of the diet.

As shown in Table 3-1, the average daily protein intake of Pemba women is 67.51 ± 9.66 g, accounting for 17.42 ± 1.32% daily energy absorption, which is slightly higher than the proportion of Spanish women's protein energy supply ratio (17.3 ± 1.6%). The average fat intake was 45.46 ± 8.75 g, accounting for 11.73 ± 0.96%, which was significantly lower than the proportion of Spanish women's fat energy supply ratio (35.4

± 6.3%). The average carbohydrate intake was 274.6 ± 39.92 g, accounting for 35.4 ± 6.3%. The ratio was 70.86 ± 9.53% daily energy absorption, which was significantly higher than that of Spanish women (47.4 ± 6.5%). As for the Pemba children, the average daily protein intake was 20.31 ± 1.97 g, accounting for 15.73 ± 1.48%, the fat intake was 14.69 ± 1.66 g, accounting for 11.38 ± 1.17%, the carbohydrate intake was 94.1 ± 3.32 g, The proportion is 72.89 ± 8.64%.

Tab. 3-1 Analysis of three main nutrient intake of healthy women and children on Pemba Island

Nutrients	Pemba women	Pemba children
protein/g	67.51 ± 9.66 （17.42 ± 1.32%）	20.31 ± 1.97（15.73 ± 1.48% ）
fat/g	45.46 ± 8.74 （11.73 ± 0.96%）	14.69 ± 1.66 （11.38 ± 1.17%）
carbohydrates/g	274.6 ± 39.92 （70.86 ± 9.53%）	94.1 ± 3.32 （72.89 ± 8.64%）

3.3.2 Composition of gut microbiota in women and children from Pemba Island

As shown in Figure 3.1, at phylum level, the four most abundant microbe in the guts of Pemba women were Firmicutes (46.90 ± 3.58%), Bacteroidetes (31.12 ± 3.76%), Proteobacteria (17.73 ± 1.89%), and Actinobacteria (1.79 ± 0.20%). At genus level, the dominant bacterium is *Prevotella 9*, with a relative abundance of 23.66 ± 3.63%, the other top ten genera were *Succinivibrio* 12.54 ± 4.95%, *Faecalibacterium* 10.17 ± 1.31%, *Agathobacter* 3.03 ± 0.67%, *Ruminococcaceae* UCG-002 2.61 ± 1.33%, *Roseuria* 2.36 ± 0.41%, *Prevotella 2* 2.13 ± 0.62%, *Alloprevotella* 2.04% ± 0.87, *Sutterella* 1.73 ± 0.50%, *Lachnospiraceae* NK4A136 group 1.70 ± 0.31%. The composition of the intestinal microbiota at the phylum level in the Spanish women group were as follows: Firmicutes (52.62 ± 3.83%), Bacteroidetes (43.52 ± 3.19%), Proteobacteria (0.89 ± 0.09%), Tenericutes (0.37 ± 0.02%). The composition at the genus level were as follows: *Bacteroides* (26.12 ± 3.51%), *Alistipes* (6.73 ± 1.12%), Ruminococcaceae_UCG-002 (5.02 ± 0.71%), *Faecalibacterium* (4.06 ± 0.93%), *Parabacteroides* (3.71 ± 0.69%), *Blautia* (2.68 ± 0.62%), *Roseburia* (2.40 ± 0.57%), *Ruminococcus*_2 (1.98 ± 0.42%), Ruminococcaceae_UCG-014 (1.91 ± 0.37%), *Agathobacter* (1.45 ± 0.27%).

As shown in Figure 3.2, the bacteria with the highest abundance in the guts of

Pemba children were Firmicutes (41.42 ± 3.87%), Bacteroidetes (30.65 ± 3.28%), Actinobacteria (15.55 ± 3.51%) and Proteobacteria (9.72 ± 2.80%). In addition, at genus level, the top ten genera were *Prevotella* 9 (21.51 ± 3.47%), *Bifidobacterium* (13.13 ± 3.03%), *Faecalibacterium* (11.98 ± 1.69%), *Lactobacillus* (5.55 ± 4.44%), *Bacteroides* (4.65 ± 1.50%), *Megasphaera* (3.11 ± 1.11%), *Collinsella* (1.56 ± 0.29%), *Agathobacter* (1.47 ± 0.57%), *Sutterella* (1.31 ± 0.26%), *Alloprevotella* (1.26 ± 0.58%). The gut composition of Australian children at the phylum level were Firmicutes (69.33 ± 4.82%), Bacteroidetes (15.58 ± 3.47%), Verrucomicrobia (6.46 ± 2.15%), Actinobacteria (4.47 ± 1.29 %). At the genus level, it was composed as follows: *Dialister* (17.72 ± 1.93%), *Eubacterium_coprostanoligenes*_group (11.22 ± 2.79%), *Bacteroides* (8.70 ± 2.53%), *Akkermansia* (7.34 ± 2,37%), *Faecalibacterium* (6.37 ± 1.92%), *Subdoligranulum* (3.78%) ± 2.51%), *Veillonella* (1.86 ± 0.47%), Christensenellaceae_R-7_group (1.22 ± 0.51%), *Bifidobacterium* (1.10 ± 0.33%), *Collinsella* (0.99 ± 0.38%).

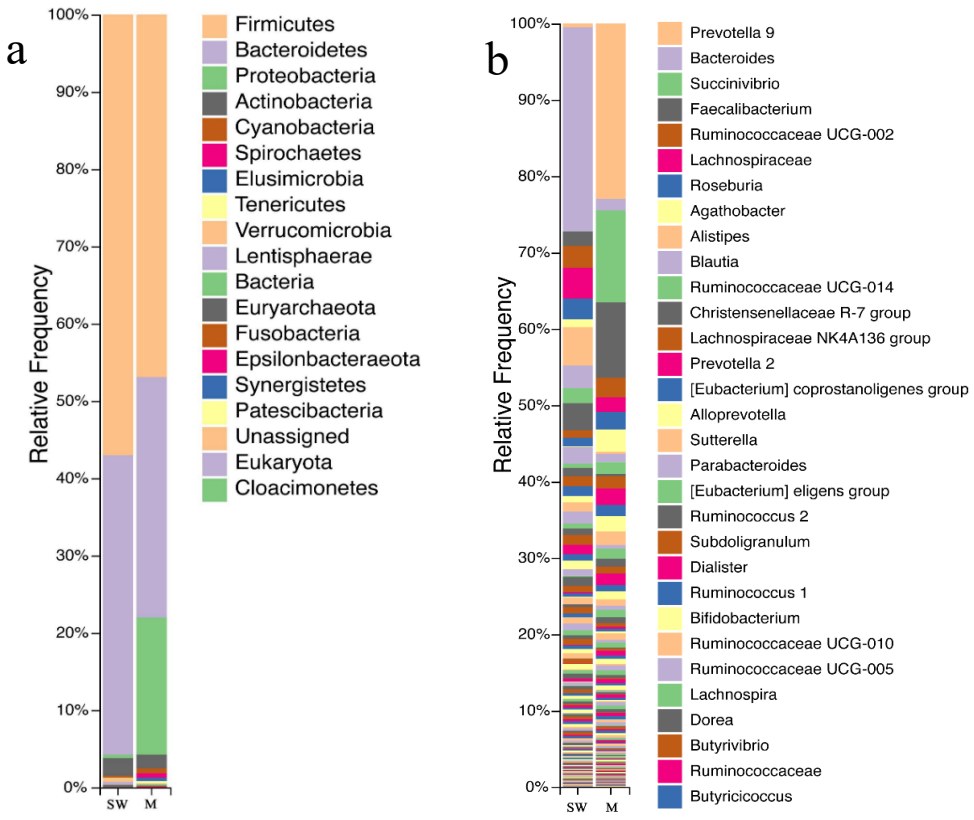

Fig.3.1 Comparison of the gut composition of healthy women from Pemba Island (M) and from Spain (SW), at phylum level (a) and at genus level (b).

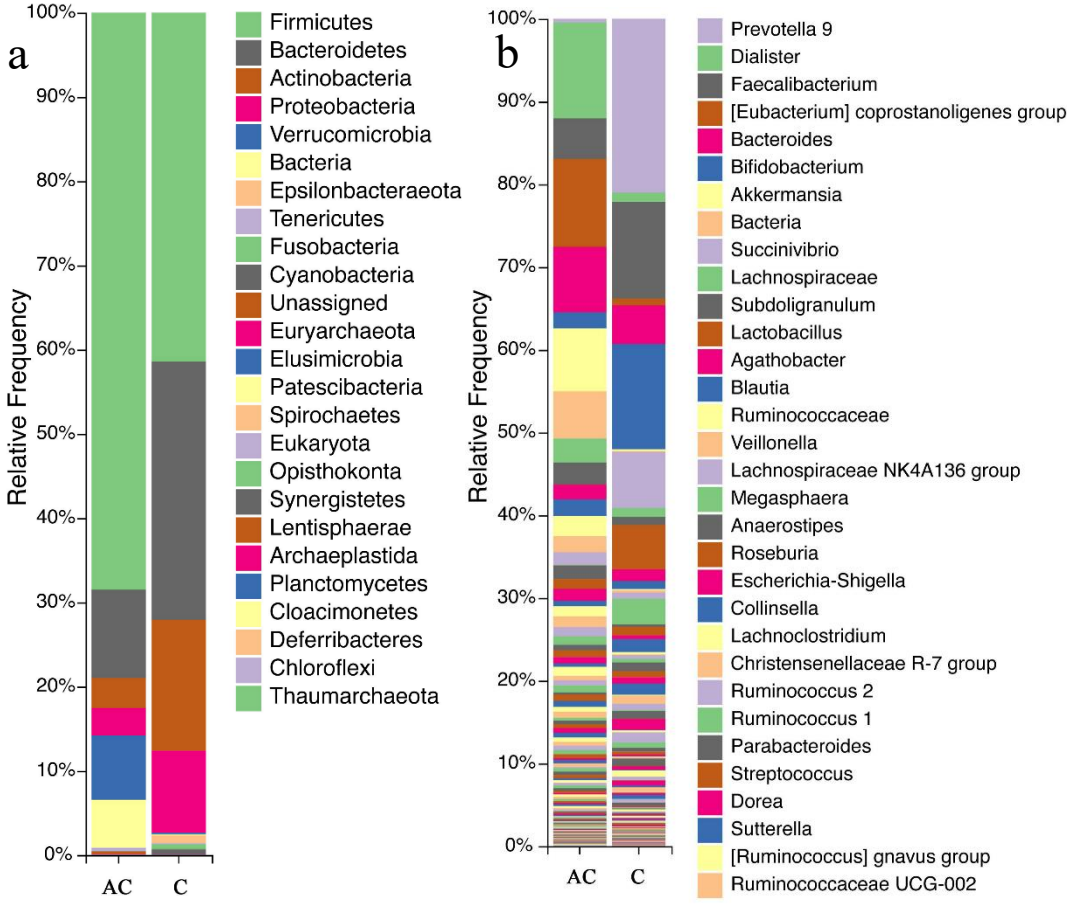

Fig.3.2 Comparison of the gut composition of healthy children from Pemba Island (C) and from Australia (AC), at phylum level (a) and at genus level (b).

3.3.3 Alpha and beta diversity of gut microbiota in women and children from Pemba Island, compared with Spanish women and Australian children

As shown in Figures 3.3 and 3.4, the observed OTUs and Faith's phylogenetic diversity index of Pemba women volunteers were significantly higher than those of Spanish women group ($P < 0.05$; $P < 0.0001$), while the Shannon index and evenness

index of Pemba women were significantly lower than those of the Spanish women group ($P < 0.0001$; $P < 0.0001$). The observed OTUs and Faith's phylogenetic diversity index of group C were higher than those of group AC ($P > 0.05$； $P < 0.01$), however, the Shannon index of Pemba children was lower than that of Australian children ($P > 0.05$), and evenness index of Pemba children was significantly lower than that of Australian children ($P < 0.05$).

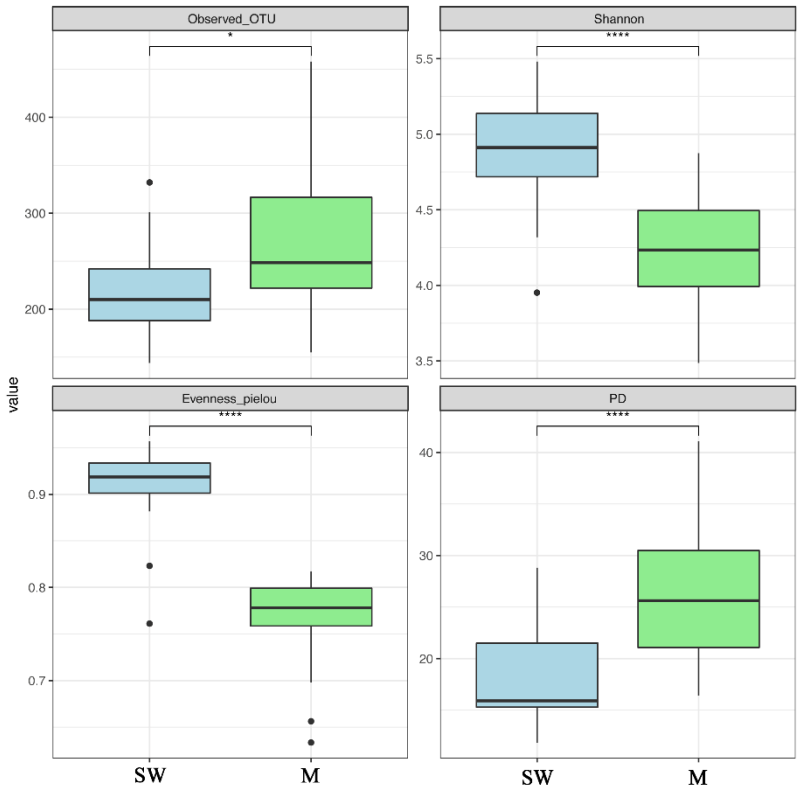

Fig.3.3 Comparative analysis of the alpha diversity indices in healthy Spanish women (SW) and mothers in Pemba (M)

Furthermore, based on unweighted principal component analysis, there were significant differences in gut microbiota structure between Pemba women and Spanish women, and between Pemba children and Australian children, respectively (Figures 3.5 and 3.6). As shown in Figure 3.5, the red square symbols represent intestinal microbiota samples from healthy Pemba women, the blue round symbols represent intestinal samples from Spanish women. And the distance between the samples represents the difference in spatial structure between the samples. The red square and blue round

samples are far from each other on the distance, and there is a tendency to cluster themselves among red samples and blue samples respectively, indicating that there is a significant difference in the gut microbiota structure between the Pemba women and the Spanish women ($p < 0.05$). Similarly, as shown in Figure 3.6, the red round symbols represent healthy Pemba children's intestinal flora samples, and the blue square symbols represent Australian children's gut samples. The red samples and the blue samples were far apart, without intersection, and there was a tendency to gather within the group, respectively, which represented a significant difference in the gut microbiota structure between Pemba children and Australian children ($p < 0.05$).

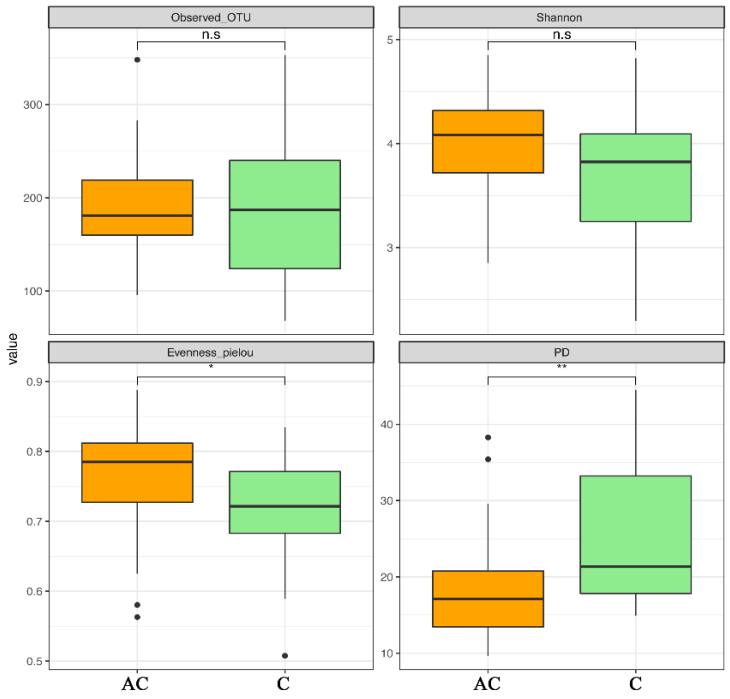

Fig.3.4 Comparative analysis of the alpha diversity indices in healthy Australian children (AC) and children in Pemba (C)

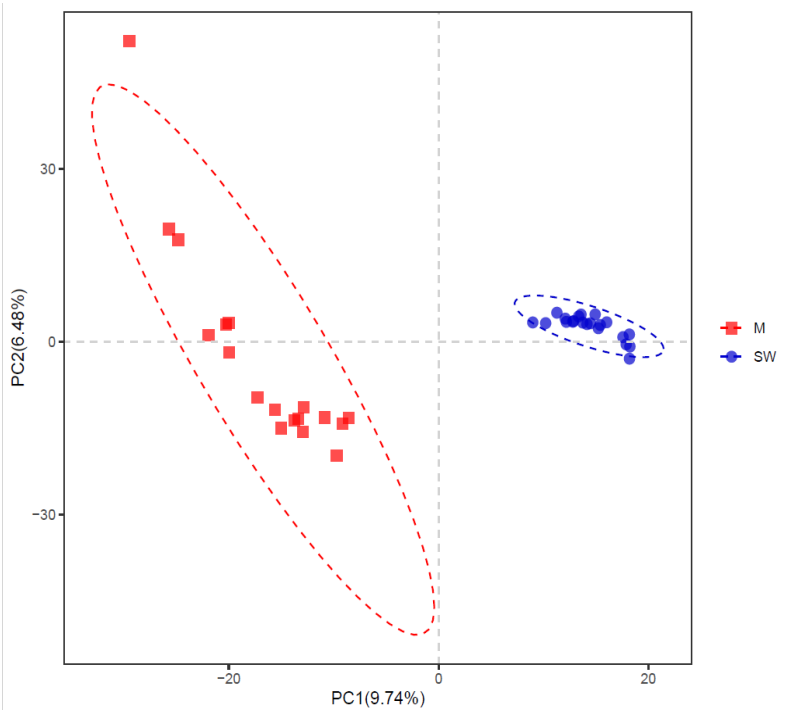

Fig.3.5 Analysis of β-diversity index in healthy Spanish women (SW) and mothers in Pemba (M)

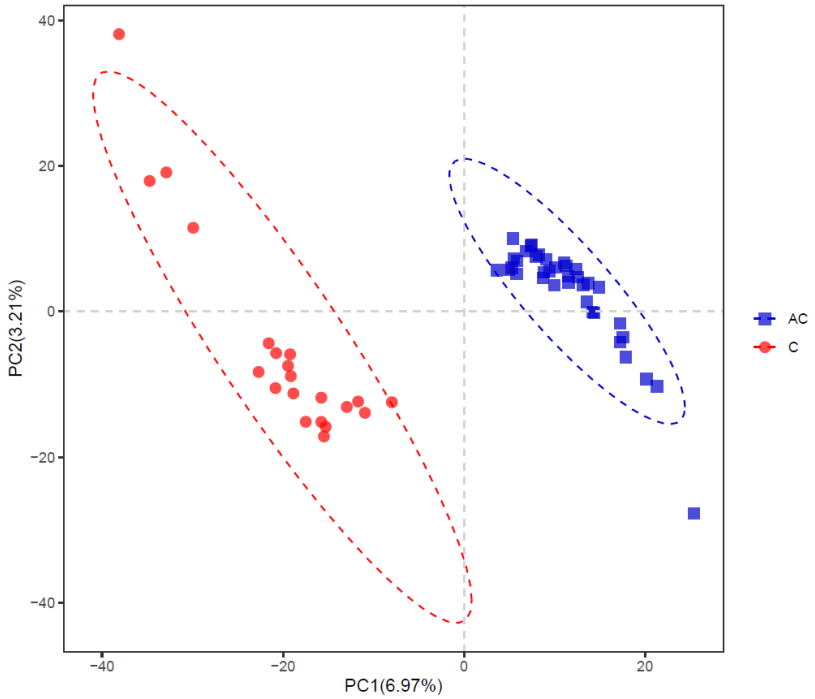

Fig.3.6 Analysis of β-diversity index in healthy Australian children (AC) and children in Pemba (C)

3.3.4 Comparison of gut microbiota profiles at genus level

As shown in Figure 3.7, *Dialister*, *Sutterella*, *Alloprevotella*, *Prevotella* 2, *Faecalibacterium*, *Succinivibrio*, *Roseburia*, and *Prevotella* 9 showed significant higher level in healthy Pemba women than in Spanish women (P <0.05). The relative abundances of *Alistipes*, *Bacteroides*, Ruminococcaceae UCG-002, and *Blautia* showed significant lower level in Pemba women than in Spanish women (P <0.05).

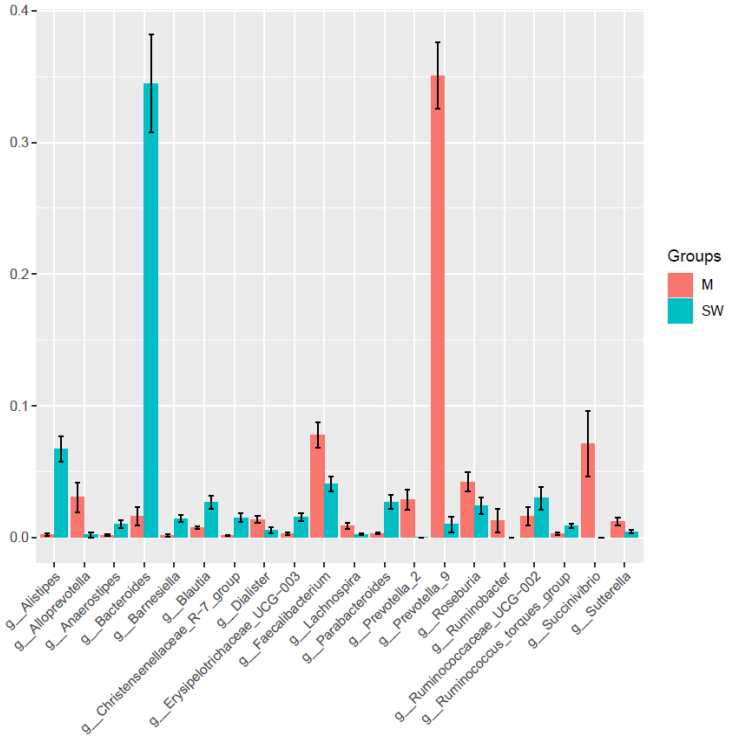

Fig.3.7 Gut microbiota distinctions between healthy Pemba Island women and Spanish women

Akkermansia, Ruminococcaceae UCG-013, *Ruminnococcus* 2 showed significant lower amount in healthy Pemba children than in Australian children. The relative abundance of *Prevotella* 9. *Lactobacillus*, *Succinivibrio*, *Megasphaera*, *Bifidobacterium* showed significant high amount in healthy Pemba children than in Australian children (Figure 3.8).

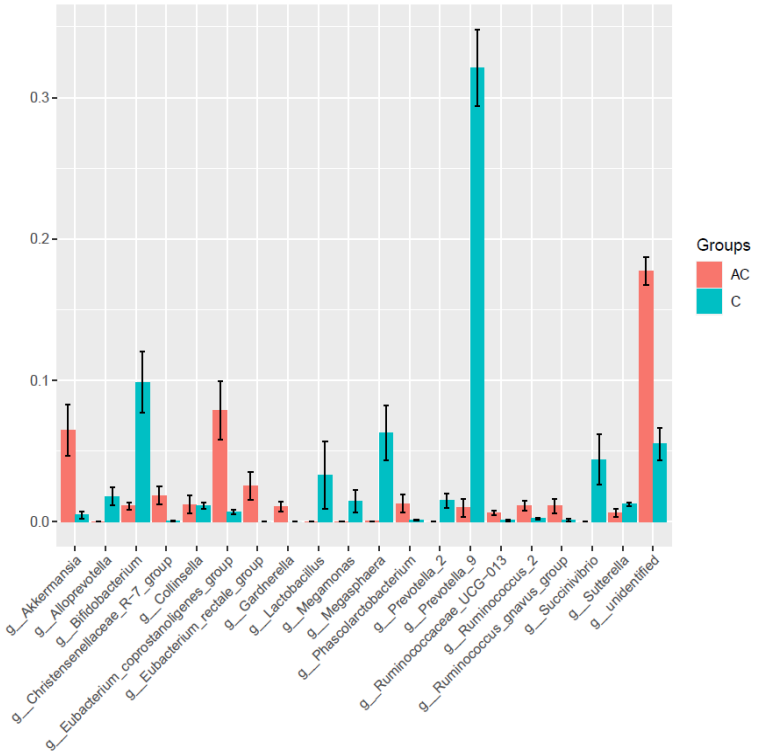

Fig.3.8 Gut microbiota distinctions between healthy Pemba Island children and Australian children

3.3.5 PICRUST analysis of functional and metabolic pathways of gut microbiota

KEGG metabolic pathways such as, “Carbohydrate metabolism”, “Glycan biosynthesis and metabolism” and “Lipid metabolism” showed significant lower abundance in healthy Pemba women than in Spanish women. While “Nucleotide metabolism”, “Metabolism of other amino acids” and “Amino acid metabolism” showed significant higher abundance in healthy Pemba women than in Spanish women (Figure 3.9).

“Xenobiotics biodegradation and metabolism” and “Lipid metabolism” showed significant lower abundance in Pemba children than in Australian children. “Metabolism of other amino acids”, “Metabolism of cofactors and vitamins”, “Nucleotide metabolism” and “Biosynthesis of other secondary metabolites” showed higher abundance in Pemba children than in Australian children (Figure 3.10).

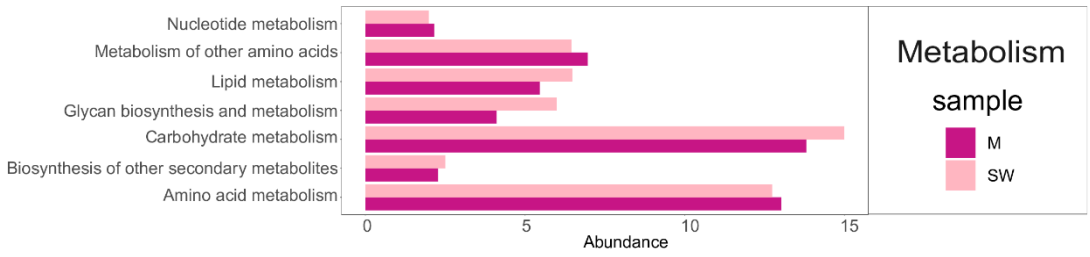

Fig.3.9 Prediction of healthy Pemba women's KEGG pathways in comparison with the prediction from Spanish women

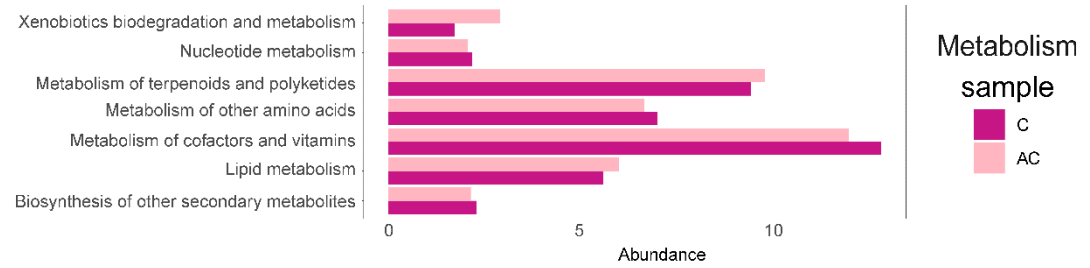

Fig.3.10 Prediction of healthy Pemba children's KEGG pathways in comparison with the prediction from Australian children

3.4 DISCUSSION

Bacteroidetes and Firmicutes were the predominant phyla in the guts of healthy Pemba women and children, Spanish women and Australian children. Bacteroidetes and Firmicutes were also found to be the predominant phyla in the gut of individuals from Denmark[186], South Korea[187], Cameroon and Botswana[164, 188]. In addition, *Prevotella* was dominant at the genus level with the largest proportion in the gut of Pemba women and children, while *Bacteroides* was the dominant genus in the gut of Spanish women. *Prevotella copri*, which is a representative strain of *Prevotella*, could regulate the catabolism of plant polysaccharides and some certain plant proteins to produce short-chain fatty acids, such as acetate and propionate [189, 190]. The representative strain of

Bacteroides is *Bacteroides vulgatus*, which is mainly responsible for the catabolism of animal-derived proteins, amino acids and fats [190, 191]. Wu et al reported that long-term high dietary fiber and carbohydrate intake was associated with the increase of *Prevotella*, while long-term intake of high-protein, high-fat, and high-calorie was associated with the promotion of *Bacteroides* in the gut [192]. High carbohydrate and dietary fiber intakes, low protein and fat intakes are the feature of Pemba women and children, and *Prevotella* is the dominant intestinal bacteria of Pemba women and children, which is in agreement with Wu' finding. Schnorr et al. reported a sex-related divergence in the gut microbiota structure of Hadza hunter-gatherers of Tanzania [180]. Although in the same lifestyle environment, gut microbiota structure of Hadza men and women were differentially adapted to their particular pattern of food consumption. Hadza women mainly feed on plant foods, which may lead to nutritional deficiencies. While Hadza men's diet consists of meat, honey, baobab, berries and tubers[163]. Diet is the most important factor which affects the gut microbiota.

Based on alpha and beta diversity analysis, there were significant differences in the composition of gut microbiota between healthy Pemba women and Spanish women, Pemba children and Australian children, respectively. The significant higher level of observed OTUs index in Pemba women indicated significant higher amounts of total numbers of gut microbes. And the significant lower levels of Shannon index and evenness index in Pemba women indicated a significant lower number of bacterial species and a lower density distribution of intestinal microbial community. Moreover, the higher level of observed OTUs index in Pemba children indicated higher total numbers of bacteria in the gut. And the lower levels of Shannon index and evenness index in Pemba children indicated a lower number of bacterial species a lower density distribution of gut microbes. In addition, principal component analysis showed significant differences in the structure of gut microbiota between Pemba women and Spanish women, and between Pemba children and Australian children, respectively. Previous studies have shown that there are significant differences in the composition and function of the gut microbiota in individuals of different ethnic origins and regions,

and diet is mainly responsible for the differences[193-196]. The diet of Spanish women is a typical western diet, whose feature are high protein, fat and calorie intakes. There are more bacterial genera involved in protein and fat catabolism in the gut, such as *Bacteroides*.

By comparing gut microbiota profiles at genus level, *Dialister*, *Sutterella*, *Alloprevotella*, *Prevotella* 2, *Faecalibacterium*, *Succinivibrio*, *Roseburia* and *Prevotella* 9 all displayed significant higher level in healthy Pemba women than in Spanish women. Among these bacteria, *Prevotella* 2, *Prevotella* 9, *Roseburia* and *Succinivibrio* were able to degrade complex carbohydrates. Furthermore, *Roseburia* could produce large amounts of butyric acid through fermentation and degradation of carbohydrates. Butyric acid has an anti-inflammatory effect and could maintain gut homeostasis. *Faecalibacterium* could also produce butyric acid and other short-chain fatty acids through fermentation of dietary fiber, which improves the intestinal barrier function and contributes to the health of the host. Carbohydrate-degrading bacteria such as *Prevotella* 9 and *Succinivibrio* were also observed to be in significant higher amount in healthy Pemba children than in Australian children. Moreover, beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* showed significant higher levels in Pemba children too. *Lactobacillus* and *Bifidobacterium* could suppress pathogen colonization in mucosa and help to prevent antibiotic-associated diarrhea [197], *Clostridium difficile*-related colictis, rotavirus-associated diarrhea and inflammatory bowel disease [198, 199].

We observed that there were analogous differences in predicted functional pathways between the gut microbiota of Pemba women and of Spanish women, and between that of Pemba children and of Australian children, by PICRUSt2 analysis. KEGG pathway “Lipid metabolism” showed significant decreased abundance both in Pemba women and children compared with Spanish women and Australian children respectively. “Nucleotide metabolism” showed significant increased abundance in Pemba women and children compared with the counterpart women and children. Considering that the proportion of fat energy supply in the three major nutrients supply system is much smaller in Pemba women and children, it is associated with the

significant decreased abundance of “Lipid metabolism” KEGG pathway correspondingly. At the same time, the abundance of *Bacteroides* which is related to the metabolism of animal protein fat showed lower level. De Filippo et al. also reported differences in the gut microbial composition between African children and Italian children. In the diets of African children, the intakes of protein and fat were significantly lower than those of Italian children, while the intake of fiber was significantly higher. At the same time, the abundance of "lipid metabolism" and "amino acid metabolism" functional pathways in African children were significantly lower than those in Italian children[179, 200]. In addition, De Filippo et al. found significant increased abundance of “Metabolism of other amino acids” pathway in African children from Burkina Faso than in Italian children[200], which is in agreement with our results. Fish, beans, and grains are the main sources of protein intake for Pemba children, therefore glutamic acid, alanine and cysteine are the mainly available beans and grains-derived amnio acids. The absence of essential amino acids in food, especially in meat, may lead to a significant increased abundance of “Metabolism of other amino acids” pathway in Pemba children than in Australian children. Because of the insufficient protein intake, African children may suffer from malnutrition and may develop Kwaschulk's disease, which is a common disease in developing countries in Africa.

3.5 CONCLUSIONS

Firmicutes and Bacteroidetes were dominant at the phylum level in the gut composition of healthy women and children from Pemba Island. *Prevotella* was dominant at genus level in the gut of healthy women and children from Pemba Island.

The gut microbial structure of healthy women and children from Pemba Island were significantly different from those of Spanish women and Australian children respectively.

PICRUST prediction analysis showed significant lower abundances of metabolic pathway “Lipid metabolism” in Pemba women and children than in Spanish women and Australian children, respectively.

CHAPTER IV DISSECTION OF THE GUT MICROBIOTA IN MOTHERS AND CHILDREN WITH CHRONIC TRICHURIS TRICHIURA INFECTION IN PEMBA ISLAND, TANAZANIA

The content of this chapter corresponds to what it has been published as article by Chen H, Mozzicafreddo M, Pierella E, et al. Dissection of the gut microbiota in mothers and children with chronic Trichuris trichiura infection in Pemba Island, Tanzania[J]. Parasites & vectors, 14(1):62.

4.1 INTRODUCTION

Soil-transmitted helminthiases (STHs) are among the most widespread neglected tropical diseases (NTDs) in low-income populations in developing regions of Africa, Asia and the Americas. Four major nematode groups, including roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), hookworms (*Ancylostoma duodenale* and *Necator americanus*), and threadworms (*Strongyloides stercoralis*), are responsible for STHs. More than 1.5 billion people are infected by soil-transmitted helminths, and 4.98 million disability-adjusted life years (DALYs) are caused by soil-transmitted helminths worldwide [201, 202]. Chronic STHs can lead to anemia, malnutrition, asthenia, abdominal pain, diarrhea, stunted growth, and cognitive and developmental impairment [203]. These nematodes penetrate the intestinal mucosa, jeopardize the intestinal epithelium and disrupt gut homeostasis [204]. STHs have been reported to increase the abundance of potential intestinal pathobionts and disrupt the structure of the gut microbial community [205]. Currently, chemotherapeutic drugs are the global strategy to control STHs, but this approach has led to an unavoidable increase in drug resistance [206]. With the emergence of anthelmintic resistance, alternative treatments are urgently required to reach the goal of the WHO to eliminate STHs [207].

The gut microbiota has been demonstrated to play pivotal roles in host health, serving such purposes as absorbing nutrients from food, modulating metabolism,

conferring resistance to colonization by some enteric pathogens and promoting the development of the immune system [4, 208]. A growing body of research has focused on helminth–microbiota interactions: for instance, some probiotic strains of *Bifidobacterium* and *Lactobacillus* have been evaluated to modulate helminth infection *in vivo* and *in vitro* [209-212]. Moreover, the impact of low-intensity, chronic helminth infection on microbial communities has been commonly reported to increase microbial alpha diversity [213-216]. In contrast, high-intensity, acute infections have been often associated to gut dysbiosis, characterized by reduced alpha diversity and an increase in pro-inflammatory and opportunistic pathogens [217]. New perspectives of helminth therapies are proposed because helminth infection may contribute positively to gut homeostasis by promoting microbial richness and evenness in individuals with chronic inflammatory disorders [216, 218, 219]; therefore it would be of interest to investigate whether helminth-induced immune modulation is related to alterations in the microbiota. Analyses of gut microbiota-mediated modulations in the worm removal and egg production of nematodes are also of interest to suggest the use of probiotics as a promising alternative means of controlling STHs by improving human health [220].

Some investigations of the gut microbiota of humans naturally infected by STHs have been reported. Jenkins *et al*. [214] reported that *S. stercoralis* infection was associated with decreased abundance of *Bacteroides eggerthii*, *Clostridium celatum* and *Bifidobacterium bifidum* in a cohort of elderly Italian volunteers by 16S rRNA gene sequencing. Lee *et al*. [213] also found that *Bifidobacterium* exhibited lower abundance and the *Paraprevotellaceae* exhibited expanded abundance in *T. trichiura*-infected individuals in Malaysia. In this study, we compared the gut microbial community of *T. trichiura*-colonized and non-colonized individuals from Pemba Island, using 16 rRNA gene sequencing. Pemba island is highly endemic for STHs; thus, this location provides a good opportunity for characterizing the intestinal microbial community of individuals suffering chronic helminth infection. In particular, we investigated mothers and their children from two rural villages and one town on Pemba Island and identified specific associations between helminth colonization and bacterial species in mothers and

children.

4.2 MATERIALS AND METHODS

4.2.1 Experiment design of study participants

According to the results of Mini-Flotac examination in Chapter I, a total of 20 helminth-positive individuals were identified. Among these 20 helminth-positive individuals, 3 were infected with *A. lumbricoides*, 14 were infected with *Trichuris trichiura*, and 3 were infected with a mixture of *Trichuris* and *A. lumbricoides*. In this chapter, we excluded the 3 single-*A. lumbricoides*-infected indivuduals (sample code M66: 2000 eggs/gram of faeces; C46: 4000 eggs/gram of faeces; C34: 10 eggs/gram of faeces), and focused on the association between *Trichuris trichiura* infection and gut microbiota. So in this chapter, there are 56 individuals from Pemba Island, including 18 healthy mother participants without helminth infection, 11 mother participants with helminth infection (9 cases with single *Trichuris trichiura* infection and 2 cases with mixed infections of *Trichuris* and *Ascaris*), 21 healthy children without helminth infection, and 6 children with helminth infection (5 cases with single *Trichuris* infection and 1 case with mixed infections). In general, there are four groups, the mother helminth-positive group (MP), mother helminth-negative group (MN), child helminth-positive group (CP) and child helminth-negative group (CN). Detailed information were listed in Table 4-1.

Tab.4-1 Information of women and children on Pemba Island and grouping arrangement

	MP	MN	CP	CN
Subject numbers	11	18	6	21
Ages (years)	29.27 ± 4.50	31.39 ± 6.63	1.63 ± 0.50	1.81 ± 0.33
Habited village				
Vitongoji	6	4	3	6
Gombani	3	6	2	6
Chake Chake	2	8	2	9
Helminth infection status	11	0	6	0
Single *T. trichiura*	9	0	5	0
T. trichiura + *A. lumbricoides*	2	0	1	0

4.2.2 DNA extraction, PCR amplification, 16S rRNA gene sequencing

Please see 2.2.4 in chapter II.

4.2.3 Bioinformatics and Sequencing Data Analysis

Please see 2.2.5 in chapter II.

4.3 RESULTS

4.3.2 Dissection of gut microbial profiling in helminth-positive and helminth-negative individuals

The 16S rRNA gene sequencing produced a total of 1,655,316 sequences after assembly and quality filtering from 56 samples, and the average length of the sequences was 410.97 bp. Good's coverage index was greater than 99%. In total, 6,252 OTUs were observed in all four groups, the mother helminth-positive group (MP), mother helminth-negative group (MN), child helminth-positive group (CP) and child helminth-negative group (CN). Rarefaction curves of the OTUs of all samples indicated that there was sufficient data sampling and adequate sequencing depth, and the database of 16S rRNA gene sequences almost completely covered all microbial communities.

There was a significant difference in the fecal microbiota alpha diversity between the MN and MP groups. The observed OTUs, Shannon index, and Faith's phylogenetic diversity index in the MP group were significantly higher than those in the MN group, respectively (Kruskal-Wallis H-test: $H = 3.82$, $df = 1$, $P = 0.05$; $H = 4.65$, $df = 1$, $P = 0.03$ and $H = 8.53$, $df = 1$, $P = 0.003$). While the observed OTUs and Shannon index, the latter accounting for both microbial richness and evenness, in group CN were not significantly different to those in the CP group (Kruskal-Wallis H-test: $H = 3.06$, $df = 1$, $P = 0.08$ and $H = 1.36$, $df = 1$, $P = 0.24$), Faith's phylogenetic diversity index was significantly higher in the CP group than in the CN group (Kruskal-Wallis H-test: $H = 10.67$, $df = 1$, $P = 0.001$) (Fig. 4.1).

PCA based on unweighted UniFrac distances showed infection-related clustering of samples collected from mothers and children, respectively (Fig. 4.2 and 4.3). Moreover, significant infection-associated alterations in gut microbial community structure were observed in mothers (PERMANOVA: pseudo-$F = 2.05$, $df = 1$, $P = 0.003$) and children (PERMANOVA: pseudo-$F = 1.96$, $df = 1$, $P = 0.013$), which indicated differences in overall heterogeneity of microbial composition between helminth-

positive and helminth-negative groups.

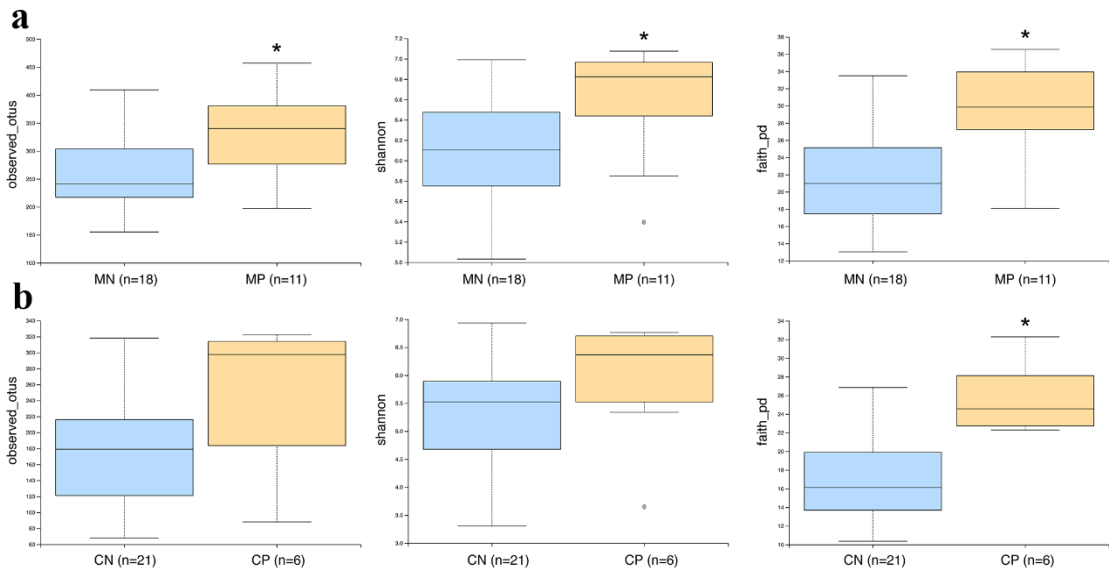

Fig.4.1 Alpha -diversity analysis of the gut microbiota

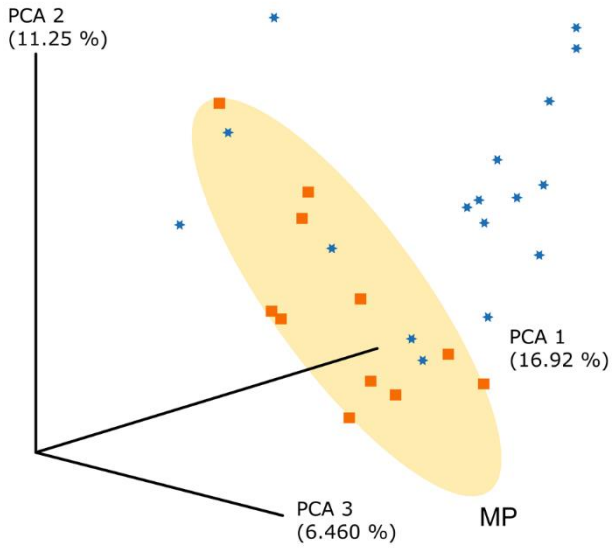

Fig.4.2 B-diversity analysis of the gut microbiota in women

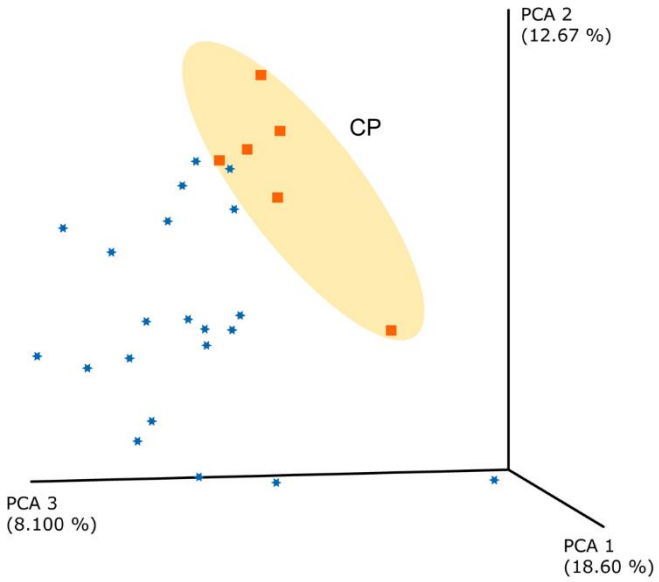

Fig.4.3 B-diversity analysis of the gut microbiota in children

4.3.3 Microbial taxa analysis

Bacterial abundances at the phylum and genus levels were analyzed and compared between infected and non-infected mothers and children. We found that Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria dominated the intestinal community in group MN at the phylum level with relative abundances of 46.90 (mean abundance) ± 3.58% (standard error), 31.12 ± 3.76%, 17.73 ± 1.89% and 1.79 ± 0.20%, respectively. In group MP, the relative abundance of Firmicutes was determined to be 57.46 ± 5.24%, while Bacteroidetes, Proteobacteria and Actinobacteria showed lower abundances of 22.31 ± 3.91%, 13.78 ± 1.41% and 1.38 ± 0.15%, respectively (Figure. 4.4). The dominant phyla in group CN and their abundances were as follows: Firmicutes (41.42 ± 3.87%), Bacteroidetes (30.65 ± 3.28%), Actinobacteria (15.55 ± 3.51%) and Proteobacteria (9.72 ± 2.80%). Group CP abundances were as shown in Fig. 4.4: Firmicutes (42.28 ± 8.22%), Bacteroidetes (29.44 ± 9.27%), Actinobacteria (15.61 ± 9.85%) and Proteobacteria (9.44 ± 3.07%) (Fig. 4.4). These results showed that the relative abundance of dominant gut bacterial populations differed between mothers and

children at the phylum level. Moreover, in mothers, helminth colonization was associated with a significant enrichment of Firmicutes and significant reductions of Bacteroidetes, Actinobacteria and Proteobacteria in gut microbial populations. In children, variations in the gut microbial composition were not significant at phylum level.

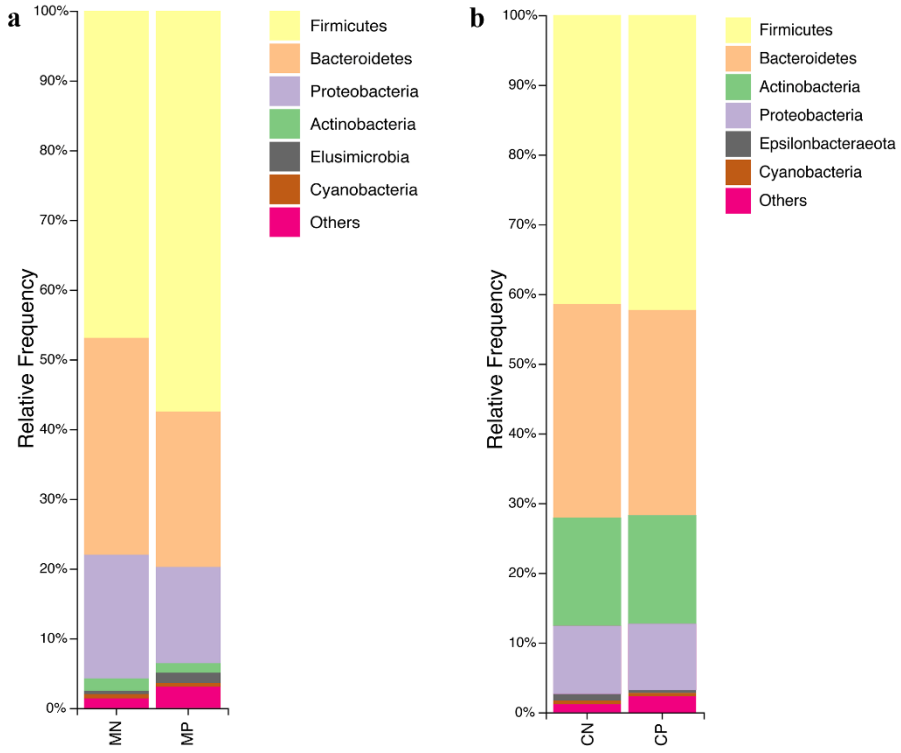

Fig.4.4 Gut composition at phylum level: (a) women and (b) children

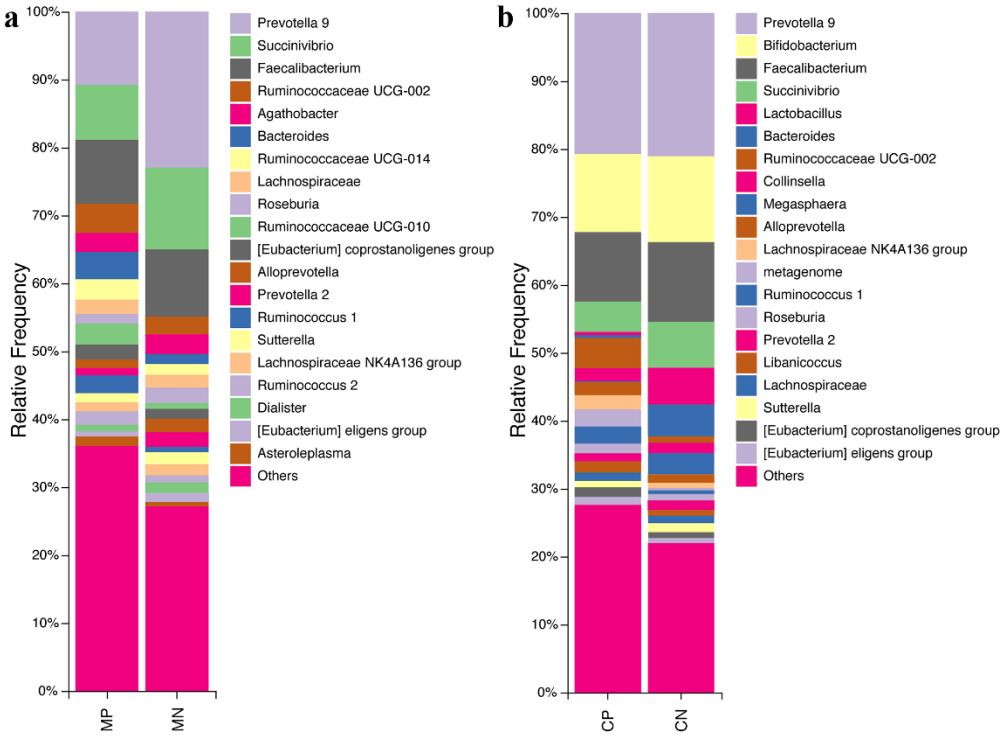

Fig.4.5 Gut composition at genus level: (a) women and (b) children

We further analyzed the microbial composition at the genus level. The 20 most abundant bacterial genera for each group are shown in Fig. 4.5 Detailed data of the abundances of each genus is shown in Appendix II. A heat map was also constructed to display microbial genera showing the largest differences in relation to helminth infection in mothers and children, respectively. *Succinivibrio* (phylum Proteobacteria) showed significantly lower abundance in both helminth-positive groups, while *Ruminococcus* 1 and *Ruminococcaceae* UCG-010 both belonging to the phylum Firmicutes showed higher abundance in both helminth-positive groups. *Bifidobacterium*, *Prevotella* 2 and *Prevotella* 9 showed significantly lower abundance in group MP than in group MN, while *Campylobacter* and *Ruminococcus* UCG-005 showed significantly higher abundance in group MP than in group MN. *Akkermansia* (phylum Verrucomicrobia), *Lactobacillus*, *Blautia*, *Bacteroides* and *Campylobacter* showed significantly lower abundance in group CP than in group CN, and

Bifidobacterium showed lower abundance in group CP than in group CN. while *Enterococcus* showed significantly higher abundance in group CP than in group CN (Fig. 4.6).

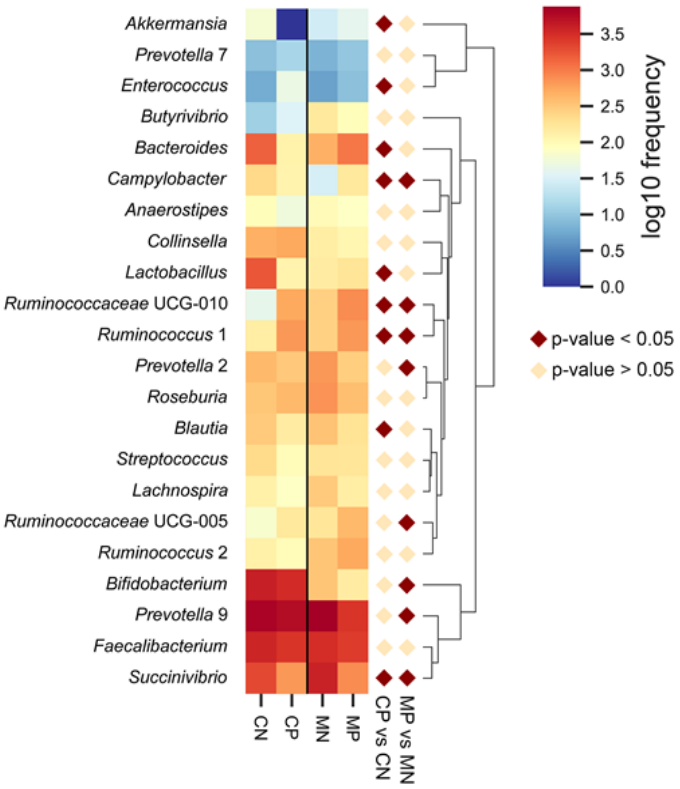

Fig.4.6 Heatmap analysis of women and children individuals

We also conducted a LEfSe analysis as additional approach to identify the significant differences in the abundance of gut microbial populations between groups MN and MP, groups CN and CP separately. The taxonomic cladogram and LDA score (threshold = 3.2) confirmed and enabled the visualization of the significant variations (Fig. 5). The LEfSe analysis showed that compared with the MN group, the abundances of *Ruminococcaceae*, *Ruminococcaceae* UCG-005 and *Lachnoclostridium* were significantly lower in group MP, while *Methanobrevibacter* and *Ruminococcaceae*

UCG-010 were significantly higher in group MP. In infected children, *Succinivibrio*, *Asteroleplasma*, *Alphaproteobacteria*, *Rhodospirillales* and *Aeromonadales* were significantly lower with respect to healthy children, while the populations of *Enterococcaceae*, *Ruminococcaceae* UCG-010 and *Enterococcus* were more enriched (Fig. 4.7 and 4.8).

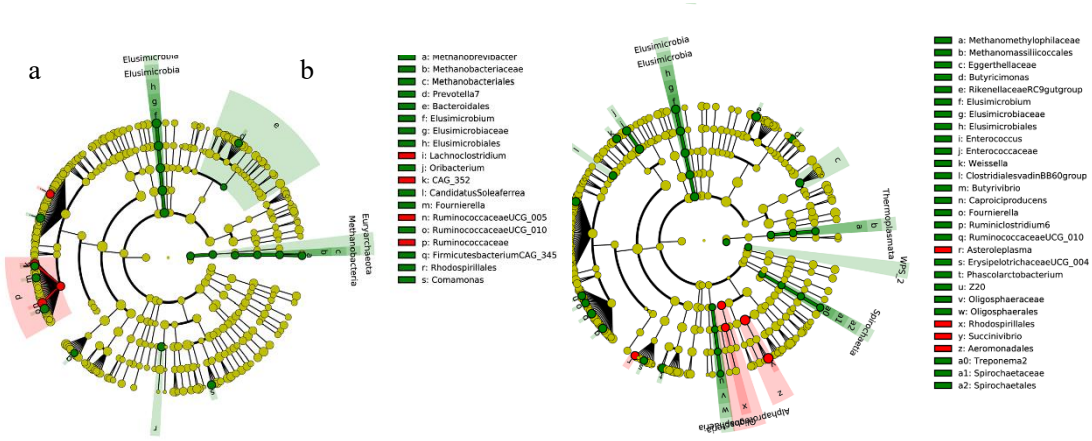

Fig.4.7 Cladograms displaying differences in mothers (a) and children (b)

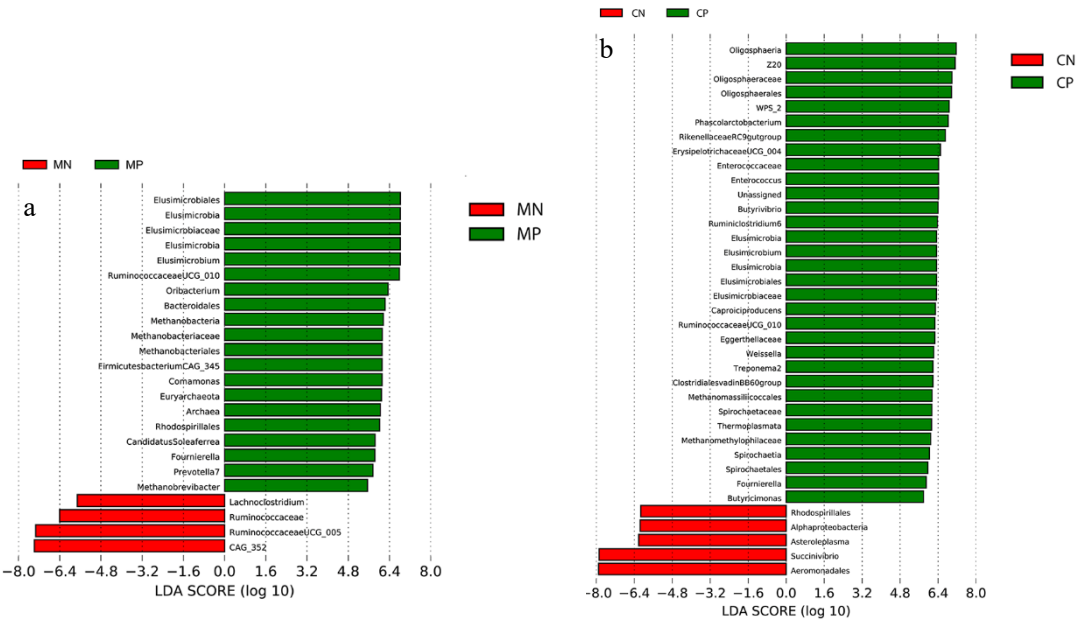

Fig.4.8 LDA score for women (a) and children (b)

4.3.4 Predicted functional KEGG pathways by PICRUSt2

We observed significant differences in predicted functional abundances between the gut microbiota of helminth-infected and non-infected mothers and children, respectively, by PICRUSt2. Several pathways such as “glycerolipid metabolism” and “sphingolipid metabolism” were less abundant in both infected mothers and children in comparison to their respective uninfected counterparts (Fig. 4.9). Infected children showed less abundance in some essential pathways, such as “cytoskeleton proteins”, “cell division”, and “apoptosis” compared to non-infected children. Infected mothers showed more abundance in pathways potentially related to pathologies, such as “*Staphylococcus aureus* infection”, “*Vibrio cholerae* infection” and “biosynthesis of ansamycins” compared to non-infected mothers (Fig. 4.9).

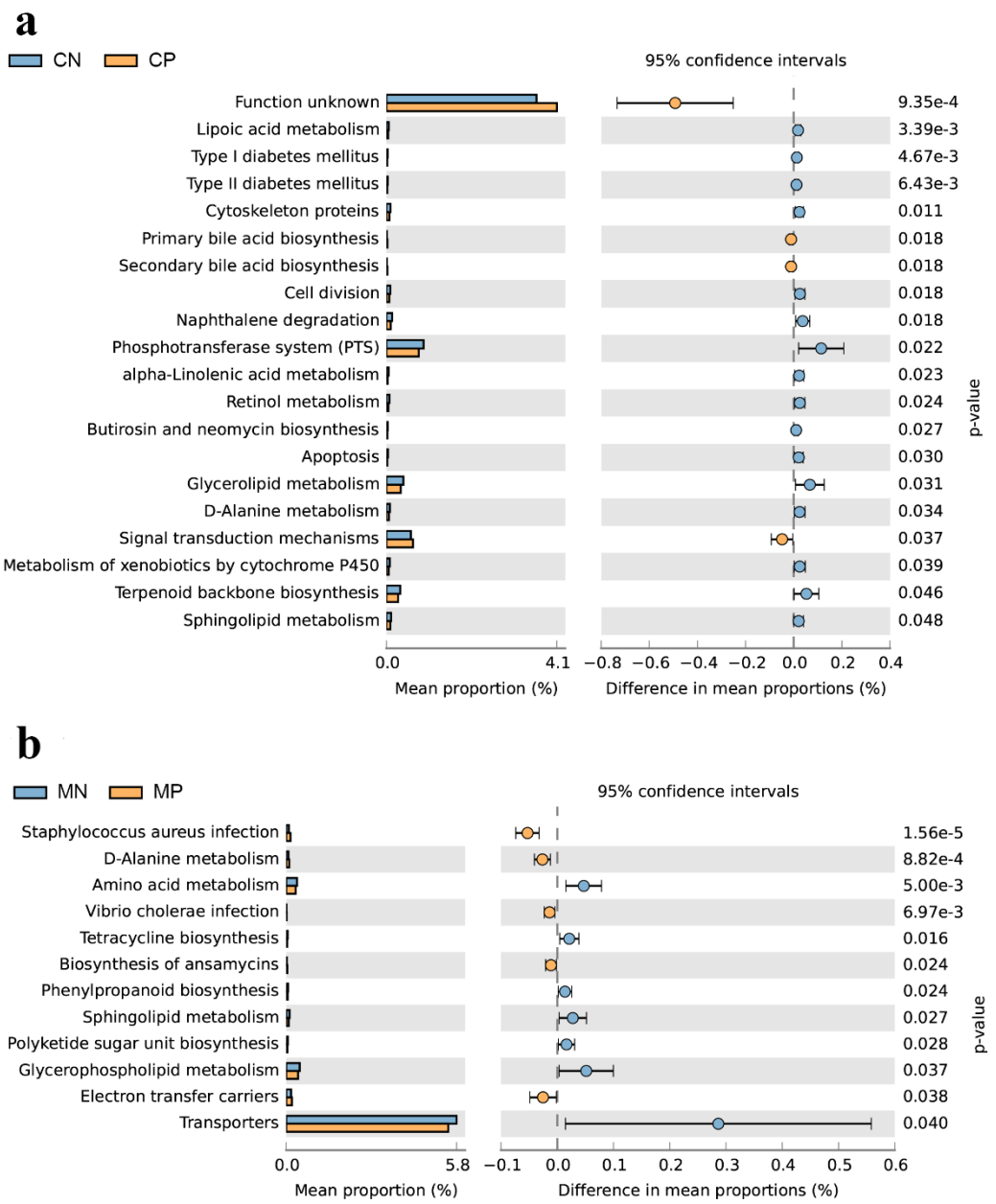

Fig.4.9 Prediction analysis of gut KEGG functional pathways in (a) women and (b) children

4.4 DISCUSSION

This study showed that *T. trichiura* infection was associated with changes in overall gut microbial composition in both mothers and children. Consistent with the findings of a previous study, Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria were the most dominant phyla in the human gut microbiota, and Firmicutes was the dominant phylum overall, with the highest abundance in all participants [213]. Alpha diversity describes the richness and evenness of the microbial samples and beta diversity provides a measure of the distance or dissimilarity between groups of samples [221]. Significant differences in both alpha and beta diversities were found between the helminth-positive and helminth-negative groups in our study, particularly in the mothers' groups. This finding is different from the previous studies. Jenkins *et al*. reported no significant changes in alpha diversity (Shannon index) and richness between helminth-positive and helminth-negative groups and significantly greater beta diversity in the helminth-positive group [222]. Additionally, Lee *et al*. reported that helminth colonization induced no significant changes in the alpha diversity of taxa; only greater richness and enrichment of Paraprevotellaceae were observed [213]. Finally, a comparison of the effects of *T. trichiura* and *A. lumbricoides* colonization on the fecal microbiota showed that only the latter was associated with a disturbed microbiota [223]. While all previous investigations support associations between helminth infection and variations in gut microbial communities, the discrepancies among these studies may be due to many factors, such as endemic areas, the type and level of helminth infections, the nutritional habits of the investigated population and the use of anthelmintic drugs [224].

Although helminth infection appeared to increase gut microbial biodiversity in both mothers and children, the abundances of well-known beneficial bacteria were lower in infected individuals, and other potentially opportunistic pathogenic bacteria showed higher abundances. The significant reductions in *Blautia* and *Bacteroides* in infected children and *Prevotella* 2 and 9 in infected mothers may limit the production of SCFAs with immunoregulatory effects to maintain mucosal homeostasis and to

regulate ulcerative colitis [225]. Since SCFAs are precursors of glycerophospholipids and glycerolipids [226], the prediction by PICRUSt2 analysis of reduced metabolism of these lipids in infected mothers and children compared with uninfected mothers and children appears consistent with our hypothesis of a reduction in SCFA production in infected individuals. Moreover, the lower amount of *Succinivibrio,* as supported by both heat map and LEfSe results, in infected children may disrupt carbohydrate digestion because *Succinivibrio* is involved in starch, hemicellulose and xylan degradation, similar to *Prevotella* [163, 181]. Finally, *Campylobacter*, which is potentially pathogenic in humans and typically represented by *C. jejuni* [227], exhibited significantly higher abundance in infected mothers. Although this variation was only validated in the heat map analysis by Student's t-test, not supported by the LEfSe algorithm, suggesting the possibility of marginal differences, this variation cannot be ignored since global surveillance of campylobacteriosis has recently been proposed due to the increasing number of pathogenic species found in humans presenting resistance to various antibiotics [228].

The changes in infected mothers and in their children compared with the uninfected groups were similar at both the phylum and genus levels. The possibility that these similarities are due to the maternal relationship in the 4 infected mother-child pairs should be considered. However, the numbers of shared OTUs at the genus level showed great variability in each infected and uninfected pair, reflecting the peculiar characteristics of the microbiota of children [229]. Therefore, it seems unlikely that the statistically significant changes in both infected groups are due only to maternal relationships.

Although similarities were found between infected mothers and children, some differences were also observed. *Akkermansia* and *Lactobacillus* appeared to be more enriched in group MP than in group MN. However, their abundances were significantly reduced in group CP compared with group CN. *Akkermansia* spp. is considered a beneficial, protective bacterium, as it is a mucin degrader that converts mucin to SCFAs, which may mediate anti-inflammatory effects [230]. Jenkins *et al*. reported that a significant increase in *A. muciniphila* was present in rural Sri Lankan populations

infected with helminths compared with uninfected populations [222], and *T. trichiura* infection dramatically increased the production of mucins in experimental macaques [231]. Therefore, the expansion of *Akkermansia*, whose sole source of energy is mucin, in group MP was expected as a consequence of *T. trichiura* infection, as also previously reported [232], but the significantly lower abundance of *Akkermansia* in group CP compared with CN may suggest concerns for gut protection for infected children. Additionally, in group CP, the significantly lower abundance of *Lactobacillus*, which is commonly used in probiotic products based on its beneficial effects on digestion processes and ability to counteract pathogenic intestinal microbiota and promote host immunomodulation [233, 234], suggests concerns for the gut homeostasis of infected children.

The life cycles of *T. trichiura* and *A. lumbricoides* are different, and *T. trichiura* does not migrate to the pulmonary circulation through the lungs. *T. trichiura* larvae attach to the intestinal villi and develop into adult worms, which then migrate and reside in the cecum and colon. However, after migration and molting, adult *A. lumbricoides* worms colonize the upper small intestine [235]. We hypothesize that the variations in colon bacteria observed in our study are associated with *T. trichiura* infection because of the colonization of same site. *Trichuris* infection could increase the secretion of mucus, which would provide an energy source for adapted microorganisms, resulting in changes in mucosal microbial composition [231, 236, 237]. The potential role of the significant increase in the mucus colonizer *Methanobrevibacter* in group MP and *Ruminococcaceae* UCG-010 in groups MP and CP in this mechanism is of interest for further research using animal models.

The significant increase in *Elusimicrobium* in all infected individuals detected by LEfSe analysis attracted our attention. *Elusimicrobium* is an acetate producer that could maintain intestinal homeostasis [238, 239]. Since *Lactobacillus* decreased in infected children, follow-up research should also focus on this genus. A previous study demonstrated that oral supplementation with live *Lactobacillus rhamnosus* at a dose of 1×10^9 CFU/day could significantly accelerate larval removal in *T. muris*-resistant

C57BL/6 mice [240]. Furthermore, persistent *T. muris* infection notably increases the population of the genus *Lactobacillus* but causes a reduction in the populations of other bacterial species in the gut [241]. The interactions between *T. muris* and *Lactobacillus* may be mutually beneficial rather than causing the microbes to eliminate each other [242]. These findings indicated that some probiotics are friendly to humans and at the same time they may have similar beneficial effects on parasites. Therefore, on the basis of the interplay between *Trichuris* and probiotics, it can be hypothesized that the *Trichuris* therapy, already applied in a few cases [243, 244], may promote growth of "friendly" bacteria to increase gut diversity to treat human IBD.

4.5 CONCLUSIONS

Alpha and beta diversities both showed significantly increased levels in *T. trichiura*-infected individuals than uninfected individuals, particularly in the group of mother indivuals.

Potentially SCFAs-producing bacteria (*Blautia* and *Bacteroides* in children; *Prevotella* 2 and 9 and Ruminococcaceae UCG-005 in mothers) decrease in abundance in infected individuals. Potentially pathogenic bacteria, such as *Enterococcus* in infected children and *Campylobacter* in infected mothers, increased in abundance.

Bifidobacterium showed decreased abundance in both infected groups, especially in the group of infected mother indivuals, as significantly decreased abundance was observed.

Elusimicrobium displayed significantly higher abundance in all infected individuals and it may serve as a potential biomarker of *T. trichiura* infection.

CHAPTER V GUT IMMUNE MODULATION OF BIFIDOBACTERIUM BREVE ON DSS-INDUCED COLITIS IN C57BL/6 MICE

5.1 INTRODUCTION

Inflammatory bowel disease (IBD) is an intestinal mucosal immune-associated disease, including Crohn's disease (CD) and ulcerative colitis (UC)[245]. The prevalence of IBD has been continuously increasing in recent years worldwide[246]. A wealth of investigations has been conducted on the pathogenesis of IBD, however, the pathogenic mechanisms are still not quite clear[247]. Genetic factors, environment, physiological and psychological changes are generally believed to trigger the onset of IBD[248]. Currently antibiotics and immuno-modulators are utilized to control CD and UC, but they cause strong side-effects and are not feasible for long-term treatment[249]. Consequently, it is necessary and urgent to develop alternative strategies to treat the disease.

The gut microbiota is an meta-organ to play vital roles in host health[250]. Large numbers of research have been extensively performed on gut microbiota that play an important role in the modulation of intestinal homeostasis[4]. Probiotics are well-defined bacteria that have the potential to provide protection against IBD[251, 252]. Moreover, probiotics and their metabolites both exerted therapeutic effects on IBD via different mechanisms[253, 254]. *Bifidobacterium*-based probiotics have been demonstrated to exert anti-inflammatory properties *in vivo* and *in vitro*[255-257]. And *Bifidobacterium* strains have confirmed protection against dextran sodium sulfate-induced colitis in mice with improved clinical and histological parameters[258-261]. *Bifidobacterium* strains were also shown to have a stimulating effect on the intestinal intraepithelial lymphocyte subpopulations and cytokines including IL-6, TNF-α, IL-1β and IL-10[255, 262-264]. *Bifidobacterium*-based probiotics is promising to apply as an alternative therapeutic strategy to control IBD in the future.

In chapter IV, our data demonstrate that changes in gut microbial composition and structure occur in *T. trichiura*-infected individuals compared with uninfected individuals. The relative abundance of *Bifidobacterium* was significantly lower in the infected Pemba women than in the uninfected women. The relative abundance of *Bifidobacterium* was also lower in the infected Pemba children than in the uninfected children. *Bifidobacterium* has the potential regulations on IBD, which maybe a negative regulation[265-268]or a positive regulation[255, 269-271]. In this research, gut immune modulation of *B. breve* on DSS-induced colitis in C57BL/6 mice was investigated. *16S* rRNA gene sequencing and flow cytometry were utilized to explore the potential mechanisms for regulating colitis.

5.2 MATERIALS AND METHODS

5.2.1 Bacterial strains and culture conditions

The strain of *Bifidobacterium breve* was deposited in Jilin Provincial Engineering Research Center of Animal Probiotics of Jilin Agricultural University (Changchun, China). De Man, Rogosa and Sharpe medium plus L-cysteine (mMRS) was utilized to culture the bacteria at 37 °C under anaerobic condition until the optical density at 650 nm reached 1.6. Prior to administration to mice, bacterial culture was centrifuged (4000 rpm, 10 min at 4°C). The cell pellets were collected and washed three times with phosphate buffer saline (PBS, pH 7.2) solution, and then re-suspended at concentrations of 5×10^8 CFU/mL, 1×10^9 CFU/mL, 5×10^9 CFU/mL in PBS solution, respectively.

5.2.2 Animals, DSS-induced colitis and experimental design

Female C57BL/6 mice (4~6 weeks old) were purchased from Beijing Huafukang Biotechnology Company (Beijing, China). They were raised under specific pathogen-free conditions; food and water were provided ad libitum, and a 12-h light/dark cycle was maintained per day. Twenty-five mice with similar body weights were assigned equally into five groups: control group (C), DSS group (DSS), low concentration B. breve-treated group (L), middle concentration B. breve-treated group (M), high concentration B. breve-treated group (H).

The entire animal experiment lasted 14 days. All the mice were free to drink sterilized water from day -7 to day 0 and only the mice in control group were allowed to drink freely with sterilized water from day 1 to day 7. All the mice were orally administrated with different composition (0.2 ml) once a day from day -7 to 0. The mice in control group and DSS group were inoculated with 0.2 ml saline (0.9%). The mice received 0.2 ml of *B. breve* with a concentration of 5×10^8 CFU/mL in group L. The mice received 0.2 ml of *B. breve* with a concentration of 1×10^9 CFU/mL in group M. The mice received 0.2 ml of *B. breve* with a concentration of 5×10^9 CFU/mL in group H. Murine colitis was induced by adding 2.5% (w/v) dextran sulphate sodium (36,000–50,000, MP Biomedicals, USA) in the drinking water from day 1 to day 7 for all the mice in groups of DSS, L, M and H. New fresh DSS solutions were prepared and replaced daily. Mouse body weights were recorded throughout the experiment and fecal samples were collected on day 7. At the end of the experiment, all mice were humanely euthanized after serum collection from the orbital plexus. Then, colon tissues and mesenteric lymph (MLN) nodes were collected. All experimental protocols were approved by the Animal Ethics Committee of Jilin Agricultural University and were performed according to the ethical guidelines of the European Community guidelines (Directive 2010/63/EU).

5.2.3 Disease activity index assessment and severity of colitis

The severity of colitis was measured using DAI assessment, a scoring system which includes three parts: body weight loss (0–4), degree of intestinal bleeding (0–4) and stool consistency (0–4)[272]. After sacrificing the mice, the colons were removed, and the colon length was measured to indicate the disease severity. The detailed the standard of scoring system was listed in Table 5-1.

Tab.5-1 Calculated disease activity index (DAI) score

weight loss（%）	stool consistency	intestinal bleeding	score
0	Normal	Negative	0

1-5	Loose stools	Negative	1
6-10	Loose stools	Hemoccult positive (slight)	2
11-15	Diarrhea (slight)	Hemoccult positive	3
Over 15	Diarrhea (Watery diarrhea)	Gross bleeding	4

5.2.4 Histological evaluation

Intestinal colon tissues were fixed in 10% formalin and embedded in paraffin wax. The tissues were dehydrated in a series of graded alcohols for staining and then sectioned. The sections were stained with hematoxylin and eosin (H&E) and examined microscopically. The histopathology score system (0–6) was utilized to evaluate the colon mucosal architecture (0–3) and inflammatory infiltration (0–3)[273].

5.2.5 Flow cytometric analysis

Single cell suspensions were prepared by grinding gently the mesenteric lymph nodes with the plunger of syringe on 200-gauge steel mesh, and suspending the cells in phosphate balanced solution (PBS). Then harvested cells were assigned to tubes with 1.5×10^7 cells per tube and stained with the immunofluorescent antibody. Alexa Fluor 700 rat anti-mouse CD3 (× 100), FITC rat anti-mouse CD4 (× 100), Pe-cy7 rat anti-mouse IL-17A (× 30), PE rat anti-mouse CD25 (× 30), Alexa Fluor 647-APC rat anti-mouse Foxp3 (× 30) were diluted using fluorescence-activated cell sorting (FACS) buffer and prepared under opaque conditions in advance. The harvested cells were incubated with various combinations of the immunofluorescent antibodies (negative control, CD3, CD4, CD25, CD3CD4, CD4CD25). Anti-mouse CD3 and anti-mouse CD4 were used to label $CD3^+CD4^+$T cells, anti-mouse CD4 and anti-mouse CD25 were used to label $CD4^+CD25^+$T cells. After staining cell surface markers, the cells were fixed and permeabilized with a Cytofix/Cytoperm kit (BD Biosciences) following the manufacturer's instructions. The cells were then intracellularly stained with Pe-cy7 rat anti-mouse IL-17A and Alexa Fluor 647-APC rat anti-mouse Foxp3 monoclonal antibodies. Finally, the cells were washed with Perm/wash buffer and resuspended in 200 μL sterile PBS for flow cytometry detection by BD LSRFortessa™ (BD Biosciences, USA). Data were analyzed with Flow Jo software (ver7.6.1, Tree Star Inc.,

USA).

5.2.6 Determination of serum cytokines by ELISA

Blood samples were collected from the orbital plexus of mice in each group. Serum samples were obtained after a centrifugal operation at 1500 rpm for 30 min. Then the serum samples were stored at - 80 ℃ for further detection. The levels of IL-10, TNF-α, and IL-1β were determined utilizing commercial ELISA kits (R & D Systems, Minneapolis, USA) according to the manufacturer's instructions.

5.2.7 Fecal microbial community analysis by 16S rRNA sequencing

Please see 2.2.4 and 2.2.5 in chapter II.

5.2.8 Statistical Analyses

One-way ANOVAs were utilized to analyze the differences between different groups in this chapter. GraphPad Prism (version 5.0) were utilized. All results are expressed as the mean ± standard error (SE). P-values of <0.05 were considered significant. Statistical significance is indicated as *P < 0.05, **P < 0.01 and ***P < 0.001.

5.3 RESULTS

B. breve ameliorated colitis symptoms, body weight loss and colon length shortening

From day 0 to day 7, during the DSS challenge, the body weight, fecal bleeding, stool consistency and diarrhea were recorded daily. As shown in Figure X, the body weights of the mice in DSS group and *B. breve*-treated groups were all consistently reduced since day 4, and B. breve-treated mice in group L, M and H showed an improvement in weight loss compared with the mice in DSS group. Prevention of *B. breve* could ameliorate the weight loss induced by DSS in mice model, and low centration of B. breve exerted the best protective effects to improve the body weight loss. At the same time, symptoms of stool consistency and fecal blood were also relieved in the *B. breve*-treated mice. The DAI score showed a significant increased level in the mice of DSS group than that in the control group. What's more, compared with the mice in DSS group, the DAI scores of *B. breve*-treated mice in the model group

(L, M and H) all showed significantly lower values (Figure 5.1).

In murine colitis model, intestinal disease severity is typically associated with colon length shortening. As shown in Figure 5.2, the colon length of the mice in control group was the longest, and the colon length of the mice in the DSS group was the shortest. The colon lengths of the DSS group and the model group (L, M and H) were all significantly decreased compared with the control group respectively. And the colon length of the model group was significantly increased compared with the DSS group. Administration of *B.breve* could improve the shortening of colonic intestinal segment in mice with DSS induced-colitis and the low concentration of *B.breve* exerted the best protective effects (Figure 5.2).

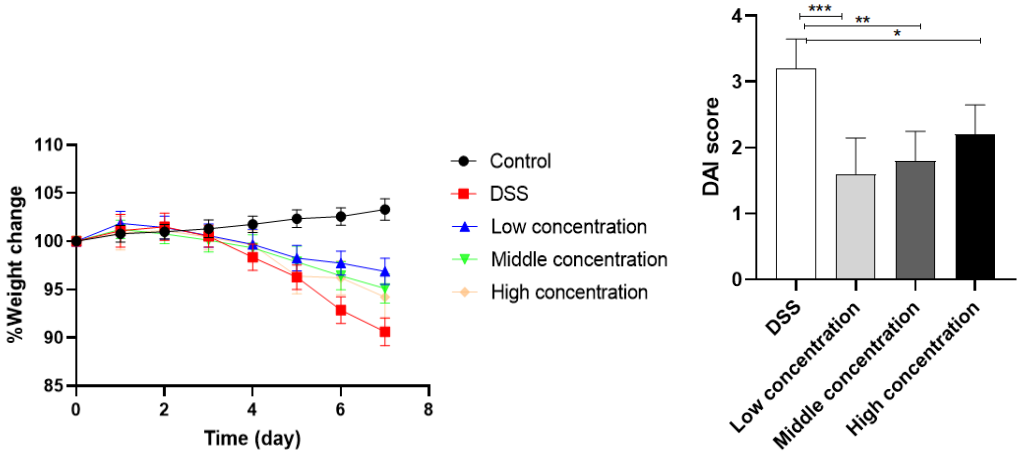

Fig.5.1 Weight changes and DAI score of the mice after DSS treatment

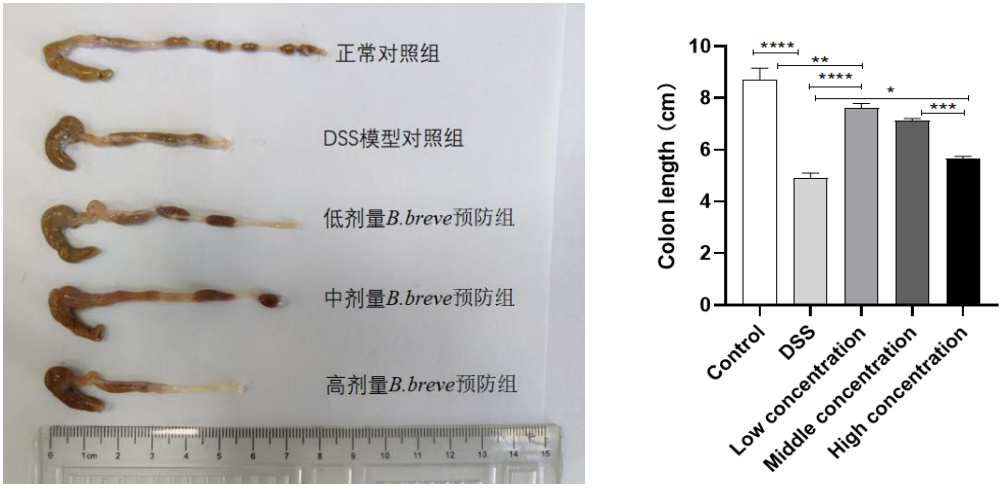

Fig.5.2 Colon length of the mice after DSS treatment

Histological analysis of the colon.

H&E staining was used to evaluate the colon histopathological damage. The colon mucosal structure of mice in control group was intact. The villi and the crypt structure were normal. No inflammatory cell infiltration or necrosis were observed in control group. The gut mucosal structure of mice in DSS group was incomplete. Submucosal edema and hemorrhage, inflammatory cell infiltration, goblet cells loss, crypt and epithelial injury were all observed in DSS group. The histopathology injury score of DSS group was the highest. Compared with the mice in DSS group, *B. breve*-treated mice in group L, M and H showed ameliorated colon tissue damage, mild inflammatory cell infiltration, reduced crypt and epithelial injury. Moreover, histological scores of group L, M and H were all significantly lower than that of DSS group, respectively ($p < 0.05$). And the histopathology injury score of group L was the lowest (Figure 5.3).

Flow cytometric analysis

As shown in Figure 5.4, significant lower levels of IL-17^+ were observed in $CD3^+CD4^+$ T cells in MLN in control group than that in DSS group ($p < 0.0001$). Significant higher levels of IL-17^+ were also observed in $CD3^+CD4^+$ T cells in model groups (L, M and L) than control group respectively ($p < 0.01$; $p < 0.001$; $p < 0.0001$). Compared with DSS group, lower levels of IL-17^+ were also observed in $CD3^+CD4^+$ T cells in model groups (L, M and L), particularly in group L and M ($p < 0.05$; $p < 0.05$). *B.breve* could inhibit the secretion of $CD3^+CD4^+$IL-17^+T in mice to suppress intestinal inflammation.

As shown in Figure 5.5, significant lower levels of $Foxp3^+$Treg were observed in $CD4^+CD25^+$ T cells in MLN in control group than that in DSS group ($p < 0.0001$). Significant higher levels of $Foxp3^+$Treg were also observed in $CD4^+CD25^+$ T cells in model groups (L, M and L) than control group respectively ($P< 0.001$; $P< 0.01$; $P < 0.05$). Compared with DSS group, higher levels of $Foxp3^+$Treg were also observed in $CD4^+CD25^+$ T cells in model groups (L, M and L), particularly in group L ($p < 0.05$). *B.breve* could facilitate the secretion of $CD4^+CD25^+Foxp3^+$Treg cells in mice to suppress intestinal inflammation.

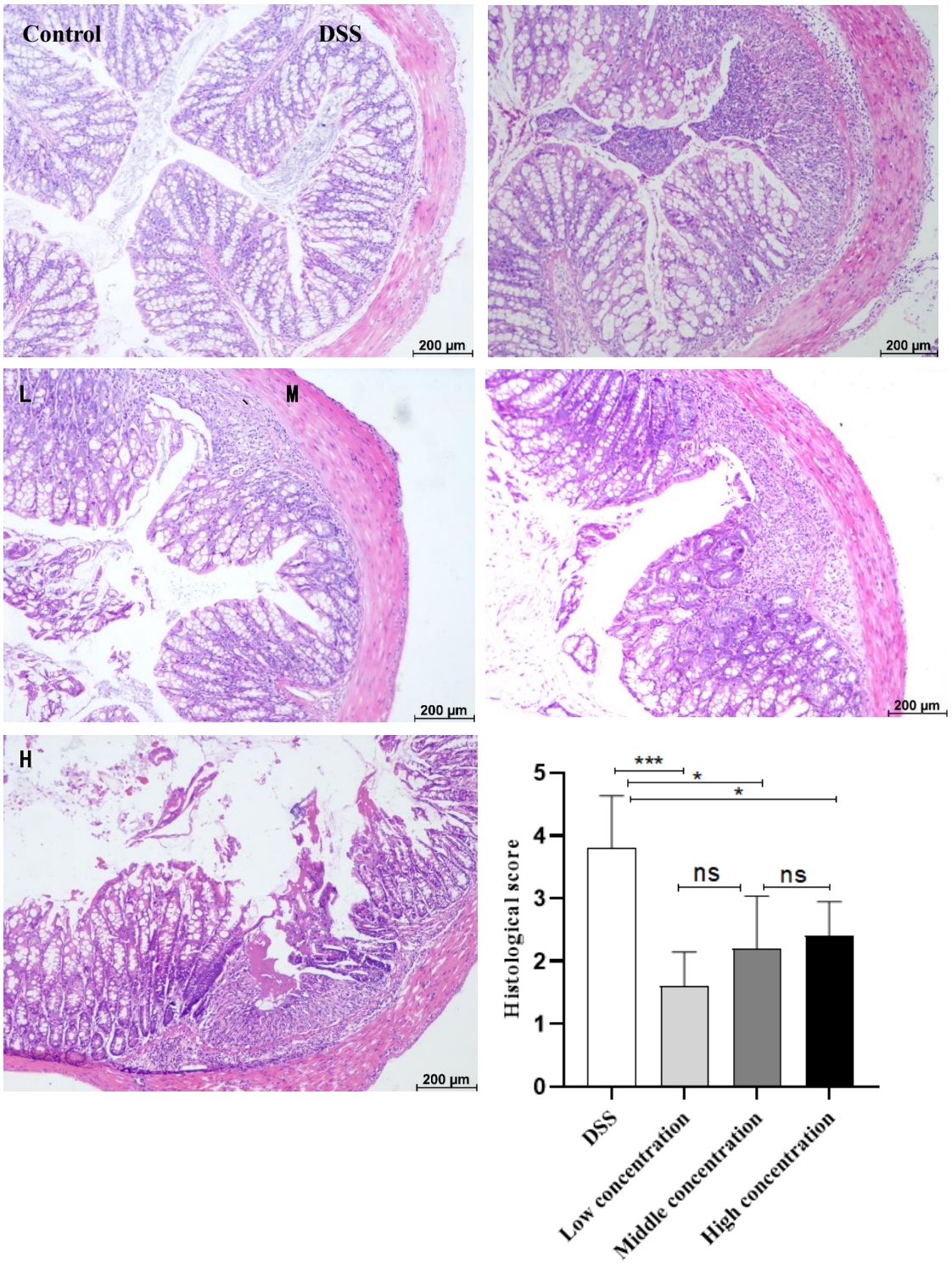

Fig.5.3 Pathological sections and histological scores of colons in mice

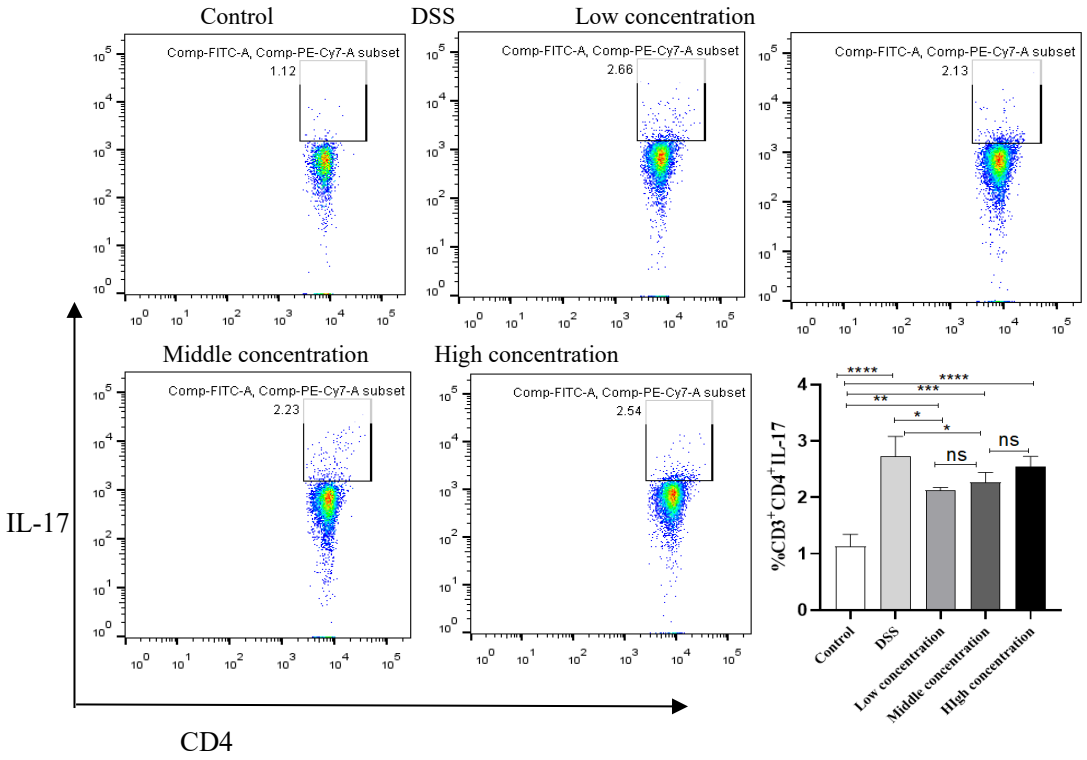

Fig.5.4 Expression of IL-17-producing T cells in the MLN

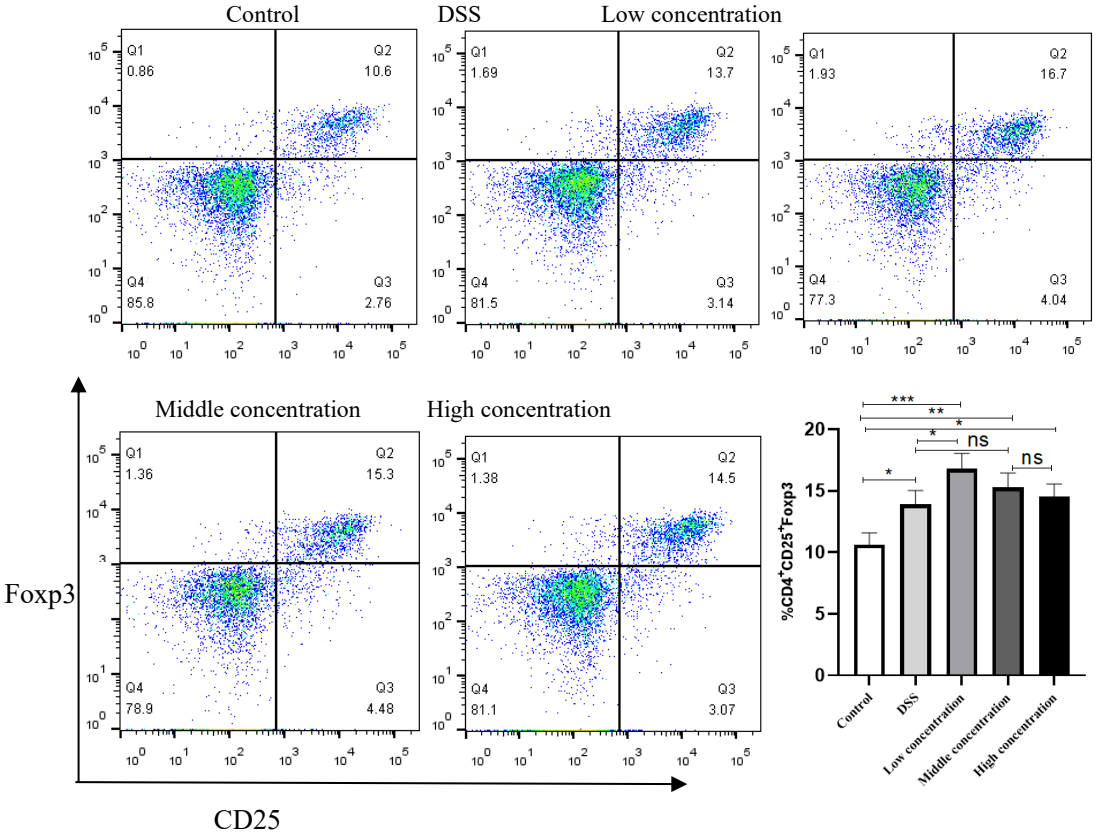

Fig.5.5 Expression of Foxp3-producing Treg cells in the MLN

B. breve regulated the serum cytokine expression

Pro-inflammatory cytokines such as TNF-α, and IL-1β, and anti-inflammatory cytokine IL-10 were used to assess the systemic level of inflammation. As shown in Figure 5.6, the levels of TNF-α and IL-1β in control group were significantly lower than those in the DSS group ($P<0.0001$; $P<0.0001$). The levels of TNF-α and IL-1β in model group (L, M and H) were also lower than those in the DSS group respectively, especially in L group ($P < 0.05$; $P < 0.001$). Besides, compared with the control group, serum levels of IL-10 in the DSS group were significantly higher ($p < 0.001$). Compared with the DSS group, the levels of IL-10 in model group (L, M and H) were also higher, particularly in L group ($P < 0.05$). Administration of *B.breve* could inhibit the secretion of pro-inflammatory cytokines, promote the secretion of anti-inflammatory cytokines.

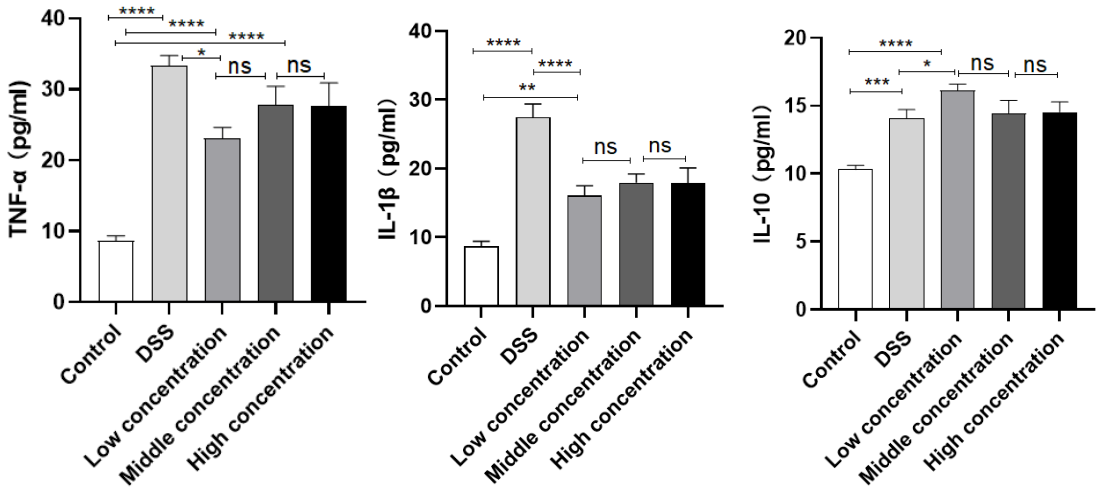

Fig.5.6 Levels of TNF-α, IL-1β and IL-10 in the serum

B. breve reshaped the gut microbiota community

There was no significant difference in the fecal microbiota alpha diversity between the control group and the DSS group, between the DSS group and the L group, respectively. However, the Shannon indices in the DSS group and the L group were both lower than that in the control group ($P > 0.05$; $P > 0.05$). Compared with the DSS group, the Shannon index in the L group increased ($P > 0.05$). Administration of low concentration of *B. breve* could increase the microbial species biodiversity in the mice

with DSS-induced colitis (Figure 5.7).

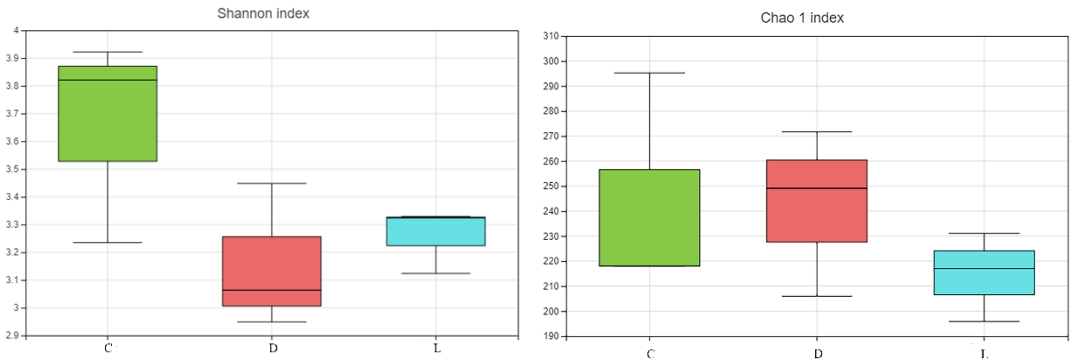

Fig.5.7 Alpha-diversity analysis of the gut microbiota

Microbial abundances at the phylum and genus levels were analyzed and compared among the three groups. We found that Bacteroidetes and Firmicutes dominated the intestinal community in the mice. In control group, the relative abundance of Bacteroidetes and Firmicutes were 37.89 (mean abundance) ± 2.72% and 45.83 ± 3.53% (standard error). Bacteroidetes (78.93 ± 5.73%) and Firmicutes (16.87 ± 2.13%) were determined in the DSS group and Bacteroidetes (61.74 ± 4.79%) and Firmicutes (25. 39 ± 2.49%) were in the L group (Figure 5.8).

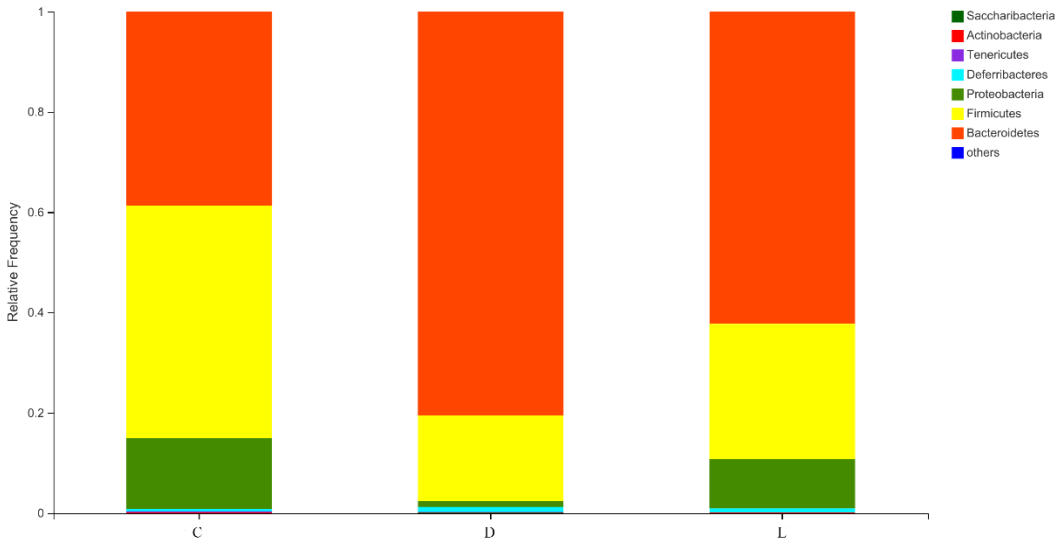

Fig.5.8 Gut composition at phylum level

At the genus level, *Bacteroides* and the no rank Bacteroidales S24-7 group were the dominant taxa in the mice. The mean relative abundance of *Bacteroides* in the control group, DSS group and L group was 10.72 ± 0.87%, 48.97 ± 4.35% and 29.56 ± 2,68%, respectively. And the mean relative abundance of no rank Bacteroidales S24-7 group in the control group, DSS group and L group was 17.25 ± 1.92%, 23.98 ± 2.12% and 23.21 ± 2.37% (Figure 5.9). At the same time, the 15 most abundant bacteria genera were compared among the three groups, the color blue refers to DSS group, the color green refers to L group and the color red refers to control group (Figure 5.10). Significant higher abundance of *Bacteroides* was detected in DSS group and L group than in the control group. Compared with the DSS group, significant lower abundance of *Bacteroides* was observed in the L group. What's more, significant lower abundances of Lachnospiraceae NK4A136 group and *Roseburia* were determined in DSS group and L group than those in the control group. Compared with the DSS group, significant higher abundances of Lachnospiraceae NK4A136 group and *Roseburia* were observed in the L group separately. Prevotellaceae UCG-001 and Lactobacillus showed the similar trends like Lachnospiraceae NK4A136 group and *Roseburia*. Lower levels of Prevotellaceae UCG-001 and *Lactobacillus* were observed in the DSS group and L group than those in the control group ($p > 0.05$). Compared with the DSS group, higher levels of Prevotellaceae UCG-001 and *Lactobacillus* were observed in the L group separately ($p > 0.05$).

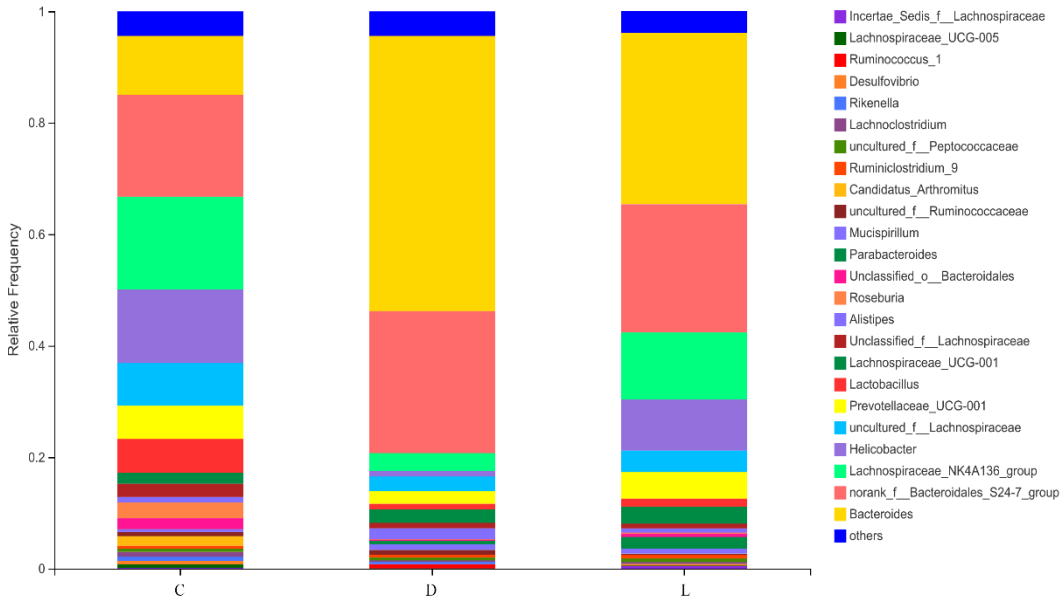

Fig.5.9 Gut composition at genus level

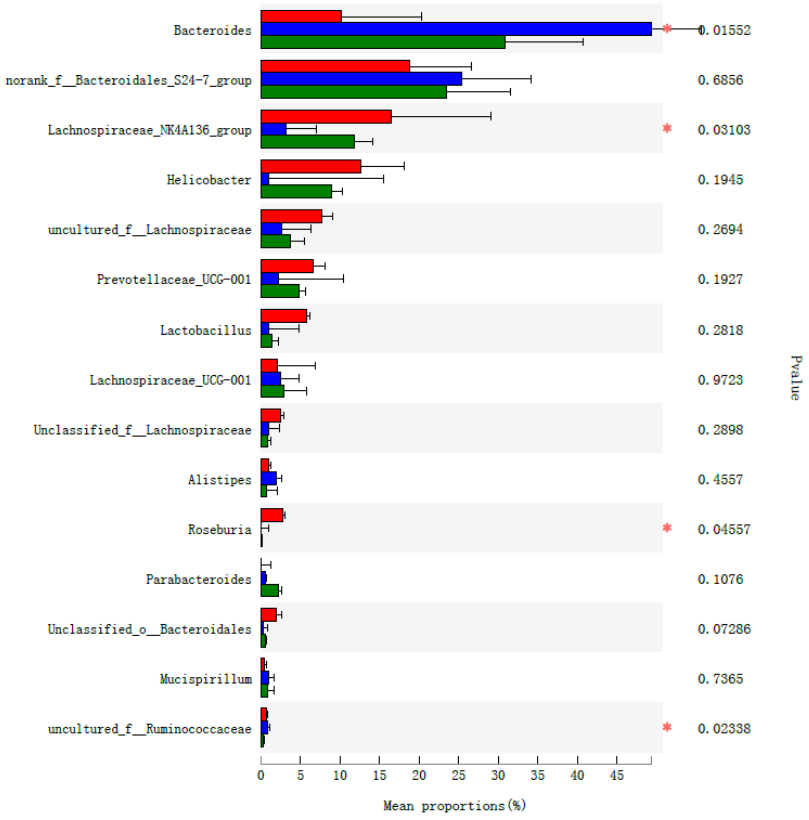

Fig.5.10 Analysis of the abundance differences in the gut microbiota

A LEfSe analysis were further conducted to identify the significant differences in the abundance of gut microbial populations among the control group, DSS group and L group. Taxonomic cladogram and LDA score diagram (threshold = 3) were prepared. Cladogram of the LEfSe analysis of the gut microbiota. The microbial compositions were compared at different evolutionary levels. The central point denotes the root of the tree of bacteria and expanded to each ring representing the next lower taxonomic level from phylum to genus. Each circle's diameter represents the relative abundance of the taxon in the gut microbial community. The LEfSe analysis showed that compared with the control group and L group, the abundance of *Bacteroides* were significantly higher in the DSS group, respectively. Compared with the control group and DSS group, abundance of *Parabacteroides* was significantly higher in the L group, respectively (Figure 5.11).

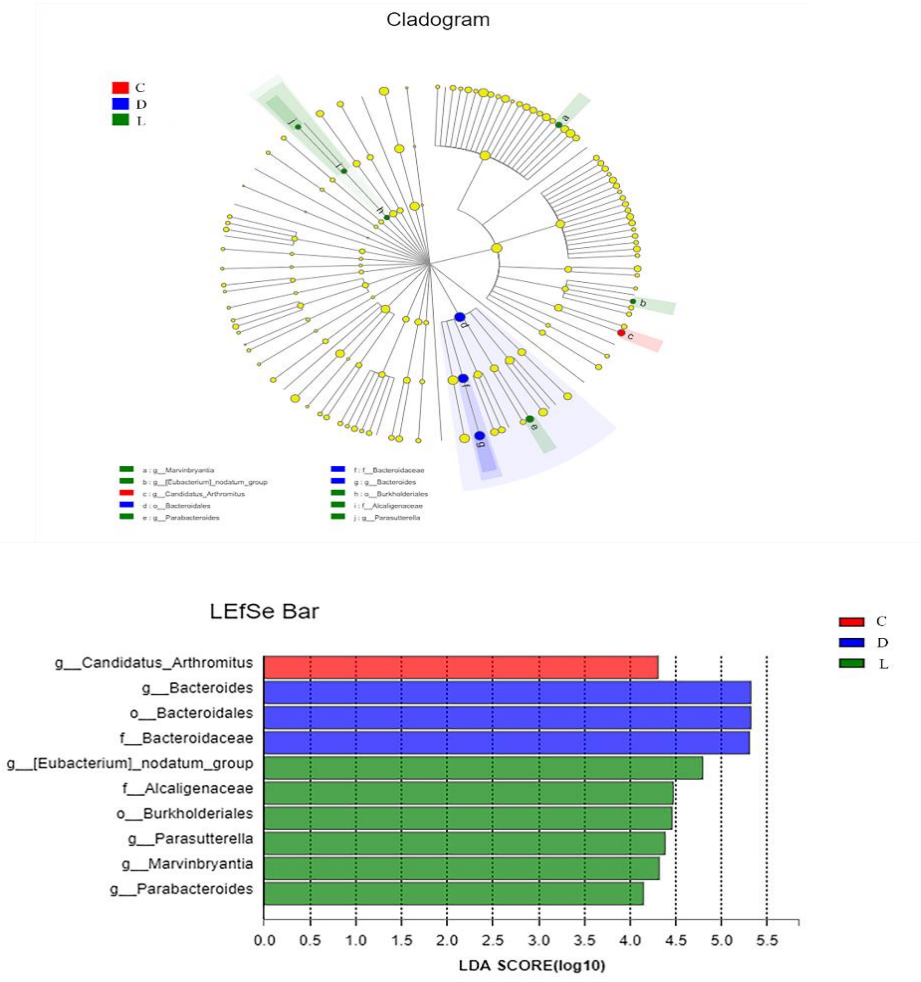

Fig.5.11 LEfSe analysis of the gut microbiota

5.4 DISCUSSION

IBD is a chronic intestinal inflammatory disease. The intestinal microbial community in IBD patients is perturbed, which is manifested as a decrease in the α-diversity of the gut microbiota, a decrease in the beneficial bacteria that produce short-chain fatty acids, and an increase in pro-inflammatory bacteria and opportunistic pathogenic bacteria[219]. A growing body of research has been conducted on the gut microbiota which has important roles in the treatment of IBD disease in recent years. Butyrate-producing bacteria-*Faecalibacterium prausnitzii* and novel probiotic-*Akkermansia muciniphila* were two milestone representative discoveries that has been applied in IBD modulation[274-276]. In chapter IV, we found that perturbations in gut microbial composition and structure occur in *T. trichiura* infected individuals compared with uninfected individuals. The abundance of *Bifidobacterium* is negatively correlated with *T. trichiura* infection. Whether the alterations in the microbiota contribute to the modulation of *Trichuris*-based Therapy in IBD is full of interest. So in this chapter, we explored the roles of immune modulation of *B. breve* on DSS-induced colitis in C57BL/6 mice.

The DSS-induced colitis model in mice is an ideal experimental model to study the pathogenesis of IBD, as the pathological damage induced by DSS is very close to the natural pathological status of the clinic. The 2.5% DSS solution did not cause the death of the experimental animals during the DSS challenge period, and the experimental animals showed clinical symptoms such as weight loss, loose stools or bloody stools, and colon length reduction. Impaired mucosal structure, hemorrhage, crypt destruction, and inflammatory cell infiltration were also observed by microscopic examination. In this study, we set up three parallel groups with different concentrations of *B. breve*. Administration of low centration, middle centration or high centration of *B. breve* all could prevent the weight loss, colon length reduction and disease severity and low centration of *B. breve* exerted the best protective effects. Our results indicated ingestion of low concentration of *B.breve* could prevent colitis in mice by regulating the expression of cytokines, reducing the secretion of pro-inflammatory cytokines (TNF-α

and IL-1β) and increasing the secretion of anti-inflammatory cytokine (IL -10), by reducing the numbers of $CD3^+CD4^+IL\text{-}17^+T$ cells, increasing the numbers of $CD4^+CD25^+$ $Foxp3^+$Treg cells, and by adjusting the composition of the gut microbiota, reducing the abundance of pro-inflammatory bacteria (*Bacteroides*). TNF-α, IL-1β and IL-17 are pro-inflammatory cytokines, which are involved in inflammatory response and immune regulation, and play a key role in pathological processes such as colitis, tissue damage, malignant tumors, organ failure and chronic inflammatory diseases[277-279]. Th2-type cytokine IL-10 is involved in the host anti-inflammatory and anti-infective immune-modulations and maintains the homeostasis of the systemic immune system and intestinal mucosal immune system[280]. Treg cells are regulatory T cells, and $Foxp3^+$ is a signature marker on the cell surface. $Foxp3^+$Treg cells could play an immunosuppressive role by secreting IL-10[281].

In this research, we also analyzed of the changes of the gut microbiota in mice after DSS challenge. We focused on the impact of low concentration of *B.breve* on DSS-induced colitis in mice. By comparing the Shannon diversity index, lower species bio-diversity was observed in the DSS group than in control group and higher species bio-diversity was observed in the L group than DSS group. Low concentration of *B.breve* improved the decrease of total bacterial species in mice after DSS-induced colitis.

Microbial taxa analysis indicated that relative abundance of Firmicutes (45.83 ± 3.53%) in the control group decreased to 16.87 ± 2.13% in the DSS group ($p < 0.05$). Firmicutes are mainly represented by Lachnospiraceae, Ruminococcaceae, Lactobacillaceae, Prevotellaceae and Clostridiaceae[274]. Lachnospiraceae NK4A136 group is an important member of Lachnospiraceae, which can produce short-chain fatty acids by fermenting carbohydrates to maintain intestinal homeostasis and is usually associated with the health status of the gastrointestinal tract[282]. *Roseburia* is a representative bacterium of Clostridiaceae and a vital producer of butyrate, which mainly colonizes in the colorectum of humans and mice[283].The abundance of important members belonging to Firmicutes, Lachnospiraceae NK4A136 group (Lachnospiraceae), *Roseburia* (Clostridiaceae), Prevotellaceae UCG-

001(Prevotellaceae) and *Lactobacullus* (Lactobacillaceae) all showed a DSS-associated reduction. In addition, the relative abundance of Bacteroidetes (37.89 ± 2.72%) in the control group increased to 78.93 ± 5.73% in the DSS group ($p < 0.05$). The relative abundance of *Bacteroides* in the DSS group was 3.7 times higher than that in the control group. LEfSe analysis indicated that *Bacteroides* may act as a candidate biomarker of DSS-induced colitis, which is in agreement with the previous study[284]. *Bacteroides fragilis* could invade intestinal epithelial cells and induce tissue damage, resulting in inflammatory responses. *Bacteroides vulgatus* and *Bacteroides Ovatus* were also reported to exert pro-inflammatory effects and contribute to the pathogenesis of IBD[285]. Taken together, administration of low centration of *B.breve* could adjust the gut microbial structure and recover the bio-diversity of the gut microbiota, by reducing the loss of SFACs-producing bacteria and probiotics, inhibiting the proliferation of pro-inflammatory bacteria.

5.5 CONCLUSION

According to the findings emerged from this test in mice, the administration of low concentration of *B.breve* could decrease the DAI score in mice with DSS-induced colitis, reduce colon length shortening and alleviate the pathological damage of tissue.

Moreover, the administration of low concentration of *B.breve* could regulate the cytokine expression in serum, increase the number of regulatory T cells in MLN, and reduce the number of Th17 cells to alleviate intestinal damage.

Finally, the administration of low concentration of *B. breve* could reshape the composition of gut microbial community, reduce the loss of SCFA-producing bacteria such as Lachnospiraceae NK4A136 group and *Roseburia*, inhibit the proliferation of pro-inflammatory bacteria such as *Bacteroides*, to relieve the intestinal inflammation.

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