Effects of probiotic *Lactobacillus pentosus* S-PT84 and prebiotic isomaltodextrin on microbiota population in low-grade chronic inflammation in C57BL/6 mouse model

By

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ABSTRACT

Effects of probiotic *Lactobacillus pentosus* S-PT84 and prebiotic isomaltodexrin on microbiota population in low-grade chronic inflammation in C57BL/6 mouse model

Lingzi Zong

University of Guelph, 2017

Advisor: Professor Y. Mine

This project aimed to demonstrate the effect of probiotic *Lactobacillus pentosus* S-PT84 and prebiotic isomaltodexrin (IMD) on disordered gut microflora in lipopolysaccharide (LPS)-induced low-grade chronic inflammatory mouse model. LPS administration to mice leads to increased intestinal permeability and elevated LPS, which ultimately causes metabolic endotoxemia and low-grade inflammation. Real-time quantitative polymerase chain reaction (qRT-PCR) was used to determine quantitative changes of *Bifidobacteria bifidum*, *Lactobacillus casei*, *Escherichia coli*, *Clostridium difficile*, and *Bacteroides fragilis*. Pre-treatment with *L. pentosus* S-PT84 or IMD increased the relative population expression of *B. bifidum*, *L. casei*, and *B. fragilis*, and decreased the relative population expression of *E. coli* and *C. difficile*. Moreover, probiotic *L. pentosus* S-PT84 and prebiotic IMD alleviated the disturbance of gut microflora in LPS-treated mice as the number of *B. bifidum*, *L. casei*, and *B. fragilis* increased, and *E. coli* and *C. difficile* decreased when compared to LPS-treated mice. The analysis of short chain fatty acids (SCFAs) further supported that the concentrations of acetic and butyric acids were positively correlated with probiotic *L. pentosus* S-PT84 and prebiotic IMD, as well as the number of beneficial bacteria.
This study elucidates that probiotic *L. pentosus* S-PT84 and prebiotic IMD are potent nutraceuticals, beneficial for gut microbiota improvement.
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<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td>AdipoR2</td>
<td>adiponectin receptor 2</td>
</tr>
<tr>
<td>CD</td>
<td>Crohn's disease</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DGGE</td>
<td>denaturing gradient gel electrophoresis</td>
</tr>
<tr>
<td>FID</td>
<td>flame ionisation detector</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization</td>
</tr>
<tr>
<td>FOS</td>
<td>fructooligosaccharide</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GI</td>
<td>gastrointestinal</td>
</tr>
<tr>
<td>GLP</td>
<td>glucagon-like peptide</td>
</tr>
<tr>
<td>GOS</td>
<td>galactooligosaccharide</td>
</tr>
<tr>
<td>IBD</td>
<td>inflammatory bowel disease</td>
</tr>
<tr>
<td>IEC</td>
<td>intestinal epithelial cell</td>
</tr>
<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IMD</td>
<td>isomaltodextrin</td>
</tr>
<tr>
<td>IMO</td>
<td>isomaltooligosaccharide</td>
</tr>
<tr>
<td>IκBα</td>
<td>light polypeptide gene enhancer in B-cells inhibitor</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>LGG</td>
<td><em>Lactobacillus rhamnosus</em> GG</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>matrix assisted laser desorption ionisation time of flight mass spectrometry</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MyD88</td>
<td>myeloid protein differentiation factor 88</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor kappa-light-chain-enhancer of activated B cells</td>
</tr>
<tr>
<td>qPCR</td>
<td>real time quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>TLR4</td>
<td>toll-like receptor 4</td>
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<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-α</td>
</tr>
<tr>
<td>T-RFLP</td>
<td>terminal-restriction fragment length polymorphism</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PPAR γ</td>
<td>peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>PRR</td>
<td>pattern recognition receptor</td>
</tr>
<tr>
<td>SCFA</td>
<td>short-chain fatty acids</td>
</tr>
<tr>
<td>sTNF-R</td>
<td>soluble tumor necrosis factor receptor-α</td>
</tr>
<tr>
<td>UC</td>
<td>ulcerative colitis</td>
</tr>
<tr>
<td>VLDL</td>
<td>very low-density lipoprotein</td>
</tr>
<tr>
<td>ZO-1</td>
<td>Zonula occludens-1</td>
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1. Literature Review

1.1 Gut health

The gastrointestinal tract (GI tract) is a long tube from the stomach to the anus, and is the largest digestion and immune organ of the host. The primary purpose of the GI tract is to decompose food and provide nutrients to the host. A large amount of digestion and the absorption of almost all digestive products are carried out in the small intestine. Additionally, the GI tract acts as a barrier to prevent harmful substances, such as foreign antigens, toxins, and microorganisms, from entering the host and causing infections (Duggan & Walker, 2002; Zoetendal et al., 2008). Therefore, it is important to maintain a healthy gut in order to keep the host healthy.

The intestinal mucosa, an important part of the intestinal barrier, serves as the first line of defense against pathogens. The intestinal wall is composed of four layers: mucosa, submucosa, muscularis, and serosa. Among them, the mucosa, directly contacting the lumen, is the innermost and most important layer where nutrient absorption and defense reactions occur (Nagler-Anderson, 2001). Under normal circumstances, the intestinal mucosal exerts the barrier function by selectively absorbing the nutrients, and controlling the passage of enterobacteria and macromolecular substances through the intestinal wall into organs and tissues. The ability to maintain such functions is defined as intestinal permeability (Miele et al., 2009). Many factors can influence intestinal permeability such as an altered mucus layer, modified gut microbiota, and damaged epithelium (Arrieta et al., 2006). The intestinal permeability is closely related to the intestinal mucosal structure; epithelial cells lining the mucosal surface acts as the main physical barrier between the lumen and mucosal tissues. Further, the intestinal epithelium manages the absorption of dietary nutrients, water, and other beneficial substances from the
intestinal lumen (Menard et al., 2010). Tight junctions (TJs) seal adjacent epithelial cells to prevent the passage of molecules and ions through the space between cells, playing an important role in the maintenance of intestinal epithelial barrier function. The intestinal epithelium and TJs work together as a protective barrier to regulate the intestinal permeability and maintain the mucosal barrier function (Suzuki, 2013).

Additionally, gut health is closely associated with gut microbiota. More than 95% of bacteria living in the GI tract act in a commensal or pathogenic relationship with the host depending on their reaction to intestinal changes and metabolic activity (Sekirov et al., 2010; Wallace et al., 2011). After a long period of evolution, gut microbiota has established a stable symbiotic relationship with the host. Intestinal microflora is closely related to energy harvest and storage, immune function development, and host health improvement (Clemente et al., 2012).

The intestinal flora is involved in the nutritional metabolism of the host, which digests polysaccharides, proteins, fats and other organic compounds into smaller molecules. Among them, short chain fatty acids (SCFAs) are major products of bacterial fermentation of undigested carbohydrates. SCFAs are the main energy source of colonic mucosal epithelial cells, which can protect mucosa barrier and enhance anti-inflammatory activity (Ley et al., 2005; Robles, Alonso, & Guarner, 2013). Intestinal microflora, as an autoimmune stimulator, can affect the immune regulation of the host through local immune response of the intestinal mucosa. As well, the intestinal flora can compete with pathogenic bacteria for attachment sites on the intestinal epithelial cells and protect against pathogen invasion (Fujimura et al., 2010; Wallace et al., 2011). Under normal circumstances, the beneficial bacteria and harmful flora in human intestines are in a state of equilibrium. They coexist and depend on each other to maintain the balance of human micro-ecology. However, when the balance of healthy flora is destroyed, an unusual
combination of bacterial flora appears, which is called dysbiosis (Robles, Alonso, & Guarner, 2013). In some cases, the number of certain bacteria in the gut are suppressed or substantially reduced. Many factors, such as altered food and environment, altered physiological and pathological homeostasis, and the use of drugs can affect the composition and amount of intestinal microorganisms (Rogers et al., 2016). Intestinal flora alteration leads to a damaged mucosal barrier and can induce intestinal diseases such as inflammatory bowel disease.

Overall, the GI tract has an essential role in sustaining and protecting gut health. Alterations in the GI system can lead to physiological changes in the intestinal environment, disrupting the intestinal microbial function and influencing the host’s health.

1.2 Gut Microbiota and Health

Gut microbiota contain trillions of microorganisms of which bacteria are dominant followed by fungi and viruses (Guarner & Malagelada, 2003). The distribution, diversity, and quantity of organisms vary according to different parts of the body. The highest density of microorganisms is present in the colon at \(10^{12}\) CFU/mL, with anaerobic bacteria accounting for the leading group (Harvie et al., 2017).

The composition and profile of the microbiome of each individual are unique and influenced by several factors, such as the type of birth, host genetics, dietary habit, age, and usage of antibiotics and probiotics (Sommer & Backhed, 2013). At birth, colonization of microbes in the infant’s gut begins immediately (Figure 1). The quantity and diversity are increased and influenced by maternal colonization, diet, environmental exposures, and antimicrobial therapies, which can affect the incidence of some childhood diseases (Toh & Allen-Vercoe, 2015). After initial establishment of the microbiome in these early years, the number of microbes is maintained, but composition progresses continuously in both diversity
and richness (Million et al., 2013). Moreover, during adulthood, the gut microbiota becomes more diverse and stable, but changes dramatically and shows less diversity in old age (Lozupone et al., 2012). The distribution of intestinal microbiota in normal adults is stable, mainly composed of anaerobic bacteria. Among them, *Escherichia*, *Shigella*, *Faecalibacterium*, *Bifidobacteria*, *Bacteroides*, and *C. difficile* are the dominant microbiota in the feces of normal adults (Mariat et al., 2009). Elder people’s living habits, poor diet, and attenuated gastric secretion affect the composition of intestinal microbiota. The diversity of the intestinal microbiota of the elderly decreases compared to young people, with a reduced number of beneficial intestinal bacteria such as *Bifidobacteria* and *Lactobacillus* bacteria. *Enterococci* and some opportunistic pathogens are prone to multiply, making the elderly susceptible to bacterial imbalance (Zhang et al., 2015). Also, a variety of harmful bacteria in the intestine may produce toxic substances such as indole and hydrogen sulfide, corrupting the intestinal environment and accelerating aging (Khoury et al., 2016).

Although the amount of bacteria in the gut is immense, they survive in good order and are well organized. It is estimated that the human intestinal microbiome may include 500-1000 bacteria species, but the difference between the numbers of bacteria is huge (Toh & Allen-Vercoe, 2015). Approximately 99% of the population of bacteria is formed by 30 to 40 bacteria species, while many other bacteria account for only a small proportion (Sanders et al., 2013). At present, more than 400 bacterial species have been identified, of which *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Bacillus*, *Enterococcus*, and *Enterobacter* are dominant (Table 1) (Gerritsen et al., 2011). These bacteria can be divided into symbiotic bacteria, pathogenic bacteria and conditional pathogenic bacteria depending on their different physiological functions in the gut. Symbiotic bacteria are obligate anaerobes, which, in number, account for more than
99% of the total number of bacteria in the intestinal microbiota (Haque & Haque, 2017). A small proportion of bacteria include spoilage bacteria, pathogenic bacteria, and conditional pathogenic bacteria, such as *Clostridium*, *Staphylococcus aureus*, and *E. coli* (Seksik et al., 2003). The gut health plays a major role in nutrition harvest, physiological function, bacterial infection, immune reaction, drug effect, toxic response, and the aging process of the human body.
Figure 1. Development of intestinal microflora with ageing (Adapted from Rajilic-Stojanovic et al., 2009).
<table>
<thead>
<tr>
<th>Location</th>
<th>Predominant bacteria</th>
<th>Total Count</th>
<th>Major physiological processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td><em>Lactobacilli, Streptococci, Prevotella, Enterococcus</em></td>
<td>$10^2$-$10^3$</td>
<td>Secretion of acid, Digestion of macromolecules pH 2</td>
</tr>
<tr>
<td>Jejunum</td>
<td><em>Streptococci, Lactobacilli, Bacteroides, Clostridium, Enterococcus</em></td>
<td>$10^4$-$10^5$</td>
<td>Continued digestion, Absorption of monosaccharides, amino acids, fatty acids, water pH 7-9</td>
</tr>
<tr>
<td>Ileum</td>
<td><em>Enterbacteria, Enterococcus, Bacteroides, Bifidobacteria, Peptococcus, Clostridium, Lactobacilli</em></td>
<td>$10^3$-$10^7$</td>
<td>Absorption of bile acids, vitamin B12 pH 5.5-7</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td>$10^9$-$10^{12}$</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Predominant bacterium at various locations in adult gut (Adapted from Sekirov et al., 2010)
1.2.1 Gut Microbiota Function and Metabolites

The intestinal flora has a significant relationship with the occurrence and development of certain diseases. After a long period of evolution, gut microbiota has established a stable symbiotic relationship with the host. More than 95% of bacteria living in the GI tract maintain a mutually beneficial or pathogenic relationship with the host (Sekirov et al., 2010; Wallace et al., 2011). The interaction between the GI tract and the microorganism affect host health by preventing the invasion of pathogens, maintaining the integrity of the gastrointestinal barrier, and promoting the development and maintenance of the mucosal immune system (Belkaid & Hand, 2014).

The primary metabolic function of the colonic microbiota is the fermentation of food residues that are not readily digestible (Guarner & Malagelada, 2003). The overall result of this complex metabolic activity is the restoration of energy metabolism and the increased availability of absorbed substrate to the host, with the excess energy and nutrients used for bacterial growth and proliferation (Ha, Lam, & Holmes, 2014). The daily diet contains a variety of non-digestible carbohydrates including large polysaccharides (cellulose, hemicelluloses, non-starch polysaccharides, pectin, and gums), some oligosaccharides, and sugar alcohols that elude digestion and absorption (Wallace et al., 2011). It is hard for the host to digest all the nutrients from the diet, but the genetic diversity of the microbial community provides a large number of enzymes and biochemical pathways to make up for the shortcomings of the host (Jandhyala et al., 2015). As the fiber cannot be digested in the small intestine by the host, it is mainly fermented by microbiota like Bacteroides, which can create digestive enzymes to decompose polysaccharides like cellulose and hemicelluloses (Tremaroli & Backhed, 2012).

The intestinal microbiota help develop the intestinal mucosal and systemic immune
function (Hooper, Littman, & Macpherson, 2012). The gut immune system mainly depends on the intestinal mucosal immunity. The intestinal mucosal barrier, comprised of intestinal epithelial cells, intercellular tight junctions, and bacterial biofilm, can effectively prevent the penetration of bacteria into the deep tissue of the mucosa (Purchiaroni et al., 2013). A single layer of epithelial cells at the mucosal interface helps connect the host and the microorganisms, allowing the microbial metabolites to enter and interact with the host cells, thereby affecting the immune response and disease (Hooper, Littman, & Macpherson, 2012).

The gut, as an important immune organ in the human body, regulates the differentiation, activation, and function of many immune cells, such as T cells and B cells (Round & Mazmanian, 2009). Regulatory T cells (Tregs) are prevalent in the small intestine and colon and can control the adverse effects of dietary and intestinal microbiota stimulation (Izcue, Coombes, & Powrie, 2009). The immune system induces undifferentiated T lymphocytes into Tregs cells through secretion of cytokines, thus regulating the tolerance of intestinal commensal bacteria on epithelial tissue and preventing the occurrence of autoimmune diseases (Caricilli, Castoldi, & Camara, 2014). Clusters IV and XIVa of the genus Clostridium can increase the number of Tregs cells in the colon and increase the resistance to enteritis and systemic anaphylaxis in mice (Kendal et al., 2011). B. fragilis can affect the production of Tregs. Capsular polysaccharide of B. fragilis stimulates the production of IL-10, which induces the transformation of T cells to Tregs. Intestinal microbiota can stimulate Tregs and Th17 cells simultaneously and maintain the intestinal homeostasis through various inflammatory regulation mechanisms.

1.2.1.1 Short Chain Fatty Acids

Short chain fatty acids (SCFAs) are important products created by the fermentation of undigested carbohydrates in the colon and play a major role in many aspects of the host. The
types and amount of SCFAs are mainly affected by the different kinds and quantities of fermentation substrates, the rate of degradation, the intestinal microbiota, and the physiologic state of the host (den Besten et al., 2013). *Lactic acid* bacteria mainly produce lactic acid from lactose. *Bifidobacterium* belonging to *Lactic acid* bacteria also produces acetic acid and formic acid. In addition to propionic acid, *B. fragilis* produces significant amounts of succinic acid. *Bacillus* mostly produces acetic acid, butyric acid, and lactic acid. *Clostridium* produces a considerable amount of acetic acid, propionic acid, butyric acid, and lactic acid (Wong et al., 2006). Because the composition of human intestinal flora is host specific, the ability to produce SCFAs is different. Thus, the content of SCFAs in the intestinal tract can reflect the differences in structure and activity of intestinal flora in the human body (Ewaschuk et al., 2002).

Acetic acid, propionic acid, and butyric acid are the primary short-chain fatty acids (SCFAs), accounting for about 90%-95% of the total amount. SCFA provides 10% to 15% of our daily caloric requirements for the host. Butyric acid acts as an energy source for colon epithelial cells and suppress inflammation by inhibiting the production of pro-inflammatory molecules; acetic acid and propionic acid are involved in energy metabolism in the liver and peripheral tissues, especially the muscles (Aluwong, Kobo, & Abdullahi, 2010). Acetic acid and propionic acid act as a regulator of glucose metabolism. Absorption of SCFAs may result in a hypoglycemic reaction to oral dietary glucose, a consistent response to improve insulin sensitivity (LeBlanc et al., 2017).

In addition to providing energy, SCFAs play fundamental effect on gut morphology. These effects include increasing the amount of mucus in the intestinal structure, decreasing intestinal cell permeability, deepening the crypts of the intestine, increasing vascular flow, and stimulating tissues and renewal. All these effects promote barrier integrity, as well as the
function of digestive system (De Vadder et al., 2014). Their effects on host physiology and immunity also have been revealed. SCFAs have been shown to influence the maturation and development of lymphoid tissues, such as peyer’s patches in mucosal system and lymph nodes (Canani et al., 2011). They can also maintain water and electrolyte balance, inhibit the production of pro-inflammatory factors, and reduce inflammation, all of which are beneficial for the recovery of mucosal inflammation and lessen the occurrence of colon lesions. In addition, SCFAs can inhibit colon cancer cells proliferation, differentiation, and metastasis (Figure 2) (Kasubuchi et al., 2015).
Figure 2. Complex effects of SCFAs on the regulation of host metabolic functions via different mechanisms (Adapted from Kasubuchi et al., 2015).
1.2.1.2 Amino acids

Degradation of undigested or endogenous protein by intestinal bacteria is known as putrefaction and thought to be detrimental to the host's health. The hydrolyzed products are amino acids, ammonia, hydrogen sulfide, histamine, and indole. Some of them are harmful substances, but can be absorbed by the intestinal wall and detoxified by the liver (Windey, De Preter, & Verbeke, 2012). Some studies have suggested that long-term absorption of these toxins can accelerate aging, induce cancer, and cause atherosclerosis, liver disorders, and other diseases. Further, a high protein diet or an excessive amount of spoilage bacteria in the intestinal flora can cause increases in decay products (Lalles et al., 2007).

Amino acids in the gut mainly come from protein degradation by intestinal bacterial proteases and peptidases. Amino acids in the intestinal cavity can be used as the main nitrogen source for intestinal microbiota involved in microbial protein synthesis, nucleic acid metabolism, energy supplementation and other intracellular metabolic processes (Dai et al., 2013). The microorganisms in the jejunum or ileum are capable of selective oxidation of lysine, threonine, and arginine, and provide non-protein synthesized essential amino acids to the host. Some Gram-negative bacteria, such as Klebsiella bacteria and Prevotella spp. can synthesize glutamine, leucine, isoleucine, and arginine (Dai et al., 2012). Glutamic acid, the most abundant amino acid in dietary proteins, is the main energy source of the intestinal tract. Glutamate can also be fermented by intestinal flora into butyric acid and acetic acid (Blachier et al., 2007). In addition, amino acids can be converted to free ammonia by aerobic microbial deamination in the presence of deamination enzymes. The manner and metabolites of amino acid deamination are different depending on different bacteria. Some bacteria can oxidize tryptophan and produce indole and carbon dioxide, while others can use decarboxylase to produce amines (such as histamine) and
hydrogen sulfide (Hughes et al., 2000).

1.2.1.3 Vitamins

In addition, colonic microbiota contributes to the synthesis of vitamins (Miyazato et al., 2010; LeBlanc et al., 2013). Humans cannot synthesize most vitamins, so poor eating habits can result in vitamin deficiency, making vitamin synthesis by intestinal flora is critical for health. The common microorganisms like *Bifidobacterium* and *Lactobacillus* can synthesize a variety of necessary vitamins for human growth and development, such as vitamin B, vitamin K, folate, niacin, and pantothenic acid. A study confirmed that administration of high folate producing strains, such as *B. bifidum* and *Bifidobacterium longum*, to rats and humans could increase the fecal level of folate (Pompei et al., 2007). *Lactobacillus reuteri* CRL1098 was found as the first strain to produce a cobalamin-like compound, which is an essential part of vitamin B<sub>12</sub> (Taranto et al., 2003). The study found that a sterile animal without the artificial supplement of vitamin K would suffer from coagulopathy disorders (Rossi, Amaretti, & Raimondi, 2011).

1.2.2 Gut microbiota and human diseases

With the improvement of new techniques, it has been shown that diseases are more related to intestinal microflora dysbiosis rather than the change of a single bacteria (Table 2) (Clemente et al., 2012). Intestinal microflora dysbiosis is caused by many factors, such as pathogenic microorganisms, aging, diet, antibiotics, hormones, and smoking. The ecological disorder of intestinal flora can lead to the disorder of intestinal environment and cause abnormal host metabolism, immunity, and protection. Long-term metabolic alterations, chronic inflammation, and immunological defects caused by abnormal intestinal flora may eventually lead to the development of related diseases (Zitvogel et al., 2015). More and more data show that the changes in the number and type of intestinal microflora could increase the possibility of
related diseases, though these potential associations and their mechanisms are not fully understood. Studies have shown that the disorder of intestinal flora is indeed associated not only with inflammatory bowel disease (IBD), various types of cancer, but also obesity and diabetes (Guinane & Cotter, 2013).

IBD is a chronic inflammatory intestinal disease, which includes ulcerative colitis (UC) and Crohn's disease (CD). The interaction between intestinal microbiota and mucosal immunity plays an important role in the guidance and regulation of immune response in the intestine (Chen, D’Souza, & Hong, 2013). Mucosal lesions in inflammatory bowel disease are caused by the excessive symbiosis of microorganisms and dysregulation of immunity. The abnormal microbiota reduces the complexity of the gut microbial flora and is a common cause of Crohn's disease and ulcerative colitis (Manichanh et al., 2012). With the development of a new generation of sequencing technology, it has been found that the diversity of intestinal flora in IBD patients was significantly reduced, which appears as the reduction of Firmicutes and the increase of Proteobacteria (Manichanh et al., 2006). At present, it is not clear how this disorder leads to intestinal inflammation. Studies have reported that the numbers of Clostridium phyla, especially *C. difficile*, have been markedly reduced, but the changes in *Bacteroides*, *Bifidobacterium*, *lactic acid* bacteria, and *E. coli* have been inconsistent (Cao, Shen, & Ran, 2014). Another study observed longitudinally that the number of common anaerobes such as *Bacteroides, Escherichia*, and *Lactic acid* bacteria decreased before a UC relapse, with an associated drop in gut microbial diversity (Andoh et al., 2011).

Large populations of bacteria in the intestine play an important role in the development of colorectal cancer. Compared with the healthy subjects, colorectal cancer patients have less *Bifidobacteria* but more *Clostridium* and digestive *Streptococcus* (Chen et al., 2012).
Additionally, the study found more invasive *E. coli* in colorectal cancer patients with colonic mucosal lesions and adenocarcinoma compared to colorectal cancer patients with normal colonic mucosa. This result suggests that a particular strain may cause a malignant lesion of colorectal cancer (Cuevas-Ramos et al., 2010). A number of studies have found that *Streptococcus bovis*, *Bacteroides, Clostridium*, and *Helicobacter pylori* could induce cancer (Kasumi, Yashiro, & Hayashi, 2000; Whiteman et al., 2010), while *Lactobacillus acidophilus* and *Bifidobacterium* proved to be able to inhibit the deterioration of carcinogen-induced colon cancer (Chang et al., 2012; Foo et al., 2011). Thus, the increase of pathogenic bacteria can lead to the imbalance of intestinal microflora in patients with colorectal cancer.

Obesity and diabetes are metabolic diseases accompanied by persistent low-grade chronic inflammation. Obesity has become a serious global disease threatening human health. According to WHO statistics, 12% of the world's population is obese. In recent years, the relationship between intestinal flora and obesity has attracted much attention. Ley et al (2005) first demonstrated that obesity is associated with intestinal microbiota with obese mice having fewer Bacteroides but more Firmicutes than lean mice (Ley et al., 2005). Howe et al (2013) reported that by comparing the difference in gut microbiota between diet-induced obese mice at early and late stages, they found the digestive microflora increased significantly, though the family of the Rikenellaceae and Aermonas decreased (Howe et al., 2013). Cotillard et al (2013) focused on the differences in intestinal flora between obese and non-obese individuals and found the abundance of microbial genes varied considerably between them. Obese people with less abundance of microbial genes were more likely to gain weight than obese people with higher abundance (Cotillard et al., 2013). It is thus indicated that obesity is not only related to the number and type of intestinal flora, but also to the degree of abundance. These studies illustrate
that a high-fat diet can alter the structure of intestinal flora by reversibly increasing or decreasing specific microorganisms.

Intestinal flora can affect diabetes by influencing insulin resistance, blood sugar, and metabolism. More than 3.47 billion people worldwide have diabetes, according to the WHO. Larsen et al (2010) found that there was a marked change in intestinal flora in classes and genus between people with type II diabetes and healthy people. Compared with healthy people, diabetic patients had a lower relative abundance of Firmicutes but a higher abundance of Bacteroides and Proteobacteria (Larsen et al., 2010). Weight gain and insulin resistance are closely related to the presence of Firmicutes in the gut. Firmicutes can degrade polysaccharides by converting them into more digestible substances that are better absorbed by humans and bacteria themselves, providing additional energy. However, in most cases, these polysaccharides are digested directly by mammals with low utilization (Meadows, 2011). Diabetic people with higher levels of Firmicutes showed an increased burden of blood sugar. One study showed that the use of antibiotics in the treatment of Toll like receptor 2 (TLR-2) defects reduced Firmicutes to normal levels and decreased levels of fat and serum LPS, resulting in increased insulin sensitivity.

Increasing intestinal flora can reverse insulin resistance (Cani et al., 2008). Therefore, people with type II diabetes can control the intake of sugar by modulating the amount of Firmicutes, thereby controlling the development of diabetes. Furthermore, patients with diabetes often have functional disorders. Through the protection and promotion of Bacillus and Lactobacillus for substance metabolism, especially for polysaccharides and dietary fiber, the metabolism function of the host is improved (Qin et al., 2012). Most diabetic patients are accompanied by decreased immunity and chronic inflammation. The change of microflora in the intestinal tract can be used as an inducer and modulator of the immune system, preventing immune damage caused by
pathogens and directly affecting the homeostatic function (Atkinson & Chervonsky, 2012).

Intestinal flora is considered as another human organ, which influences host metabolism, physiology, and immune system development. The abnormal microbial community can lead to chronic gastrointestinal diseases. Although the molecular technologies provide us with the tools to study gut microbiota more precisely, humans now need to more accurately define the relationship between intestinal flora and several intestinal diseases. Thus, it is necessary to understand the role of microorganisms in gastrointestinal illness, which is the basis for the eventual development of appropriate therapies.
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<tr>
<th>Disease</th>
<th>Increased Microbiota</th>
<th>Reduced Microbiota</th>
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<td>IBD</td>
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<td><em>Bacteroides uniformis</em></td>
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1.3 Chronic inflammation and its impacts on human health

When the intestinal barrier cannot prevent the substances in the lumen from entering the intestinal tissues or the balance of gut microbiota is impaired, foreign substances or gut dysbiosis can induce intestinal immunological reactions to eliminate harmful components, repair the damaged beneficial colonies, and maintain the intestinal homeostasis: this process is called inflammation.

1.3.1 General aspects of inflammation

Inflammation is a complex clinical and pathological process that assists the host with the prevention of and response to cellular insults in various parts of the body’s tissues and organs (Calder et al., 2011). At the site of inflammation, mast cells are able to recognize the invasion and release chemical mediators like histamines, which lead to increased vascular permeability and vasodilation. Therefore, more immune cells in the blood vessel can migrate to the inflamed tissue or the site of infiltration or injury. The blood rushing causes the signs and symptoms of inflammation like pain, swelling, and redness (Helmy et al., 2011). Meanwhile, the resident immune cells, such as macrophages, wander into the infected area and recognize pathogen-associated molecular patterns (PAMP) on the surface of the pathogens. They also begin to secrete a number of biologically active proteins known as cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor-α (TNF-α), which increase vascular permeability and recruit more immune cells, such as monocytes and neutrophils, from the blood vessel into the tissue. Monocytes differentiate into macrophages when they are out of the blood vessel. Macrophages and neutrophils recognize and bind to the pathogens through PAMP and engulf the invading organisms to help clean up the infection; at the same time, they also mediate tissue damage by releasing chemical mediators, free radicals, and enzymes.
Meanwhile, pro-inflammatory chemokines such as chemotactic agents are produced by immune cells in the infection or injury area, and guide the migration of immune cells to the site of inflammation following a signal of increasing chemokine concentration. Chemokine receptors on the membrane of immune cells bind to chemokines so the immune cells are attracted to the infected site to advance the inflammatory response (Baggioline & Loetscher, 2000). Once invaded substances are eliminated, immune cells will produce anti-inflammatory mediators to inhibit the inflammatory reaction, promote tissue repair, and restore the normal condition of the host (Warnberg et al., 2010).

Depending on its duration, inflammation can be divided into acute and chronic. The acute inflammation is a short-term and limited beneficial defense response, which refers to an exudative change characterized by vascular response that intravascular white cells and antibodies access through blood vessels into the site of inflammation to eliminate pathogens, dilute and neutralize the toxins, and create good conditions for the repair of inflammation (Hakansson & Molin, 2011). In the acute inflammatory response, the excessive increased concentrations of acute proteins, C reactive protein (CRP) and serum amyloid A protein (SAA) considered as sensitive acute phase reactants, can signal inflammatory diseases. Additionally, different markers, such as cytokines, in systemic alterations can also be monitored (Yamada, 2005). However, if the acute inflammation cannot eliminate the pathogens or viral infection or injury continue to happen, the persistent stimulation may turn into a chronic inflammatory state. Inflammation is a natural defence system in which the body resists injury and diseases, while chronic inflammation can be harmful. Because the continuous production of pro-inflammatory cytokines and activation of the innate immune system can attack healthy areas of the host body and lead to tissue damage (Mathur & Pedersen, 2008).
In general, chronic inflammation is histologically associated with the continuous production of pro-inflammatory cytokines and the presence of mononuclear cells, such as macrophages and lymphocytes (Gabay, 2006). Macrophages, as important white blood cells in the human body, play a critical role in antigen and the inflammation process. They produce a wide range of biologically active molecules, which contributes to both beneficial and harmful effects during inflammatory reactions. Depending on different signals they receive, they create different types of chemokines, surface markers, and metabolic enzymes (Fujiwara & Kobayashi, 2005). In the chronic inflammatory state, a large number of pro-inflammatory mediators, such as LPS and IFN-γ, exist in inflammatory sites of tissues or organs. Classically activated macrophages (M1) are activated and produce pro-inflammatory cytokines, such as TNF-α, IFN-γ, IL-6 and IL-12. On the contrary, alternatively activated macrophages (M2) regulated by anti-inflammatory cytokines, such as IL-4, IL-13 and IL-10, produce more IL-10 and TGF-β to block inflammatory responses and promote tissue repair (Heilbronn & Campbell, 2008; Hakansson & Molin, 2011; almas et al., 2012). This moderate and continuous stimulation of inflammatory factors maintains the activation of the immune system for a long time, resulting in chronic inflammation.

1.3.2 Intestinal inflammation

The intestinal inflammation contributes to the chronic inflammation. As previously described, the GI tract, as an important barrier, is beneficial to the maintenance of human health. The intestinal barrier function is closely related to intestinal permeability, which prevents the invasion of foreign substances. Once the TJs or the epithelium is impaired, the integrity of the intestinal barrier structure will be reduced and intestinal permeability will be increased. Large
luminal molecules can easily pass through the impaired TJs, in which case bacteria can translocate to different intestinal locations (Mathur & Pedersen, 2008). If the damage to the intestinal barrier continues for a long time, leaky gut may happen. A leaky gut is a condition in which large amounts of waste molecules or toxins from the gut that is not completely digested by the digestive tract can be transported through the leaky gut wall into the bloodstream and then stimulate the immune and autoimmune system, leading to intestinal chronic inflammation (Anders et al., 2013). There are many factors involved in the development of the leaky gut, such as persistent inflammatory stimulants, impaired intestinal structure, and disordered gut microbiota (Lumeng & Saltiel, 2011). Modern medical research has found that intestinal inflammation plays a significant role in the occurrence and development of several chronic diseases, such as intestinal inflammatory bowel disease (IBD), obesity, insulin resistance, asthma, Alzheimer's disease, autoimmune arthritis, and chronic fatigue syndrome (Packard & Libby, 2008).

1.3.3. Low-grade chronic inflammation

In recent years, low-grade chronic inflammation as a specific type of chronic inflammation has been defined as a nonspecific, chronic, and persistent state characterized by continuously but minorly “two to four-fold” elevation in circulating levels of pro-inflammatory and anti-inflammatory cytokines, as well as other activities of inflammatory molecules in the plasma and damaged tissues (Moutsopoulos and Madianos, 2006). Because of the only slightly increased levels of pro-inflammatory molecules, there are no obvious symptoms. The causes for the low-grade chronic inflammation are similar with that of the chronic inflammation (Gökhan S. Hotamisligil, 2006).
1.4 LPS-induced low-grade chronic inflammation

There are many animal models of low-grade chronic inflammation. One study used dinitro-benzene sulfonic acid (DNBS) instillations to induce chronic low-grade inflammation and gut dysfunction in mice (Martín et al., 2015). In the present study, LPS as endotoxin is administrated to mice to initiate low-grade chronic inflammation. There are approximately $10^{13} \sim 10^{14}$ commensal bacteria in the intestinal tract of adults, and LPS is a component of the cell wall and the central pathogenic molecule of Gram negative bacteria (Wang & Quinn, 2010). In recent years, it has been found that gut permeability to bacterial LPS is an important trigger for low-grade chronic inflammation (DeGruttola et al., 2016). LPS, containing three sections: O-antigen, nuclear polysaccharide, and lipid A (Figure 3), is beneficial for bacterial growth and survival. Lipid A acts as a pathogenic component and is highly conserved and shared with all Gram-negative bacteria (Raetz & Whitfield, 2002).
Figure 3. The basic structure of LPS. LPS is composed of three distinct units; lipid A, a core oligosaccharide, and O-antigen constituted of repeating oligosaccharide units. (Adapted from Raetz & Whitfield, 2002).
Firstly, LPS can increase intestinal permeability by activating the NF-κB signaling pathways and cause excessive inflammatory responses, such as shock, leading to acute inflammation. The innate immune receptors, also known as pattern recognition factors (PAMP), can identify conserved bacterial products (Mogensen, 2009). TLR4 plays a significant role in regulating intestinal inflammation, resisting the invasion of foreign pathogens and maintaining intestinal homeostasis. In the TLR4 signaling pathway, LPS combines with LPS binding protein and CD14 to form a complex. The interaction between the complex and the extracellular portion of TLR4 leads to the dimerization of TLR4 and signal transduction to the cell, which interacts with the adaptor protein MyD88. MyD88 can collect interleukin 1 receptor related kinase (IRAK) and activate phosphorylation of IRAK. Subsequently, IRAK binds to and activates the tumor necrosis factor-receptor association factor 6 (TRAF6) (Creely et al., 2007). Through the activation of nuclear factor, Kappa B cells (NF-κB) trigger a signal transduction cascade reaction, resulting in pro-inflammatory cytokines expression, production, and secretion, and neutrophil recruitment in infection sites (Palsson-McDermott & O'Neill, 2004). Healthy people are tolerant of intestinal commensal bacteria and LPS, while the excessive activation of TLR4 signaling pathway leads to the loss of TJ structures and immune tolerance and triggers intestinal chronic inflammation. *E. coli* and some *Enterobacter* bacteria, such as *Acinetobacter baumannii* and *B. fragilis*, have an inhibitory effect on the TLR pathway of mononuclear cells through LPS on the cell wall.

Secondly, LPS is also capable to change gut microbiota composition by disrupting the intestinal mucosal barrier and inducing bacterial translocation (Mass et al., 2008). One study found that the number of Gram-positive bacteria was significantly reduced in LPS-infused mice, especially *C. difficile* and *Bifidobacteria*, which might indicate that increased levels of LPS
could reduce some beneficial bacteria in mice and influence gut bacterial community (Festi et al., 2014). Additionally, the deficiency of Bifidobacteria may affect intestinal mucosal barrier function, since Bifidobacteria does not degrade mucosal glycoproteins like other pathogenic bacteria, they can promote better gut conditions by preventing intestinal permeability and bacterial translocation (Manco, Putignani, & Bottazzo, 2010). Furthermore, a decrease in the number of Bifidobacteria in the intestines leads to reduced production of intestinotrophic hormone glucagon-like peptide (GLP-2), damaged tight junction integrity, and increased intestinal permeability. GLP-2 produced by L cells in the gut intestine helps promote intestinal growth and barrier function through insulin-like growth factor (IGF)-1 and β-catenin pathways (Spreckley & Murphy, 2015). As a result, plasma concentration of LPS can increase due to increased intestinal permeability, changes in diversity of intestinal microbiota, and increased ratio of Gram-negative to Gram-positive bacterial (Cani et al., 2008). In addition, leaky gut permeability leads to translocation of pathological agents toward target tissues such as the blood, the liver, and the adipose deposits of arterial walls to interfere with cells from the immune system (Manco, Putignani, & Bottazzo, 2010). Therefore, LPS-gut permeability interaction can be the underlying cause of chronic low-grade inflammation due to the continuous LPS penetration into blood to activate the immune system.

1.5 Probiotics and Prebiotics

Keeping the diversity of gut microbiota and the proportion of beneficial bacteria in balance is the key to maintain the intestinal functions. Probiotics are beneficial bacteria that confer a health effect on the host (Sanz, Santacruz, & Gauffin, 2010). Most probiotic bacteria are isolated from fermented foods or healthy humans, which means it is safe because of its
compatibility between the host and provider (Butel, 2014). Probiotics promote health through several interrelated mechanisms. They compete with pathogenic bacteria to limit their growth, reducing the risk of infection. They also enhance immune responses to activate reactions to dangerous infectious organisms or suppress excessive inflammation. Additionally, probiotics strengthen epithelial barrier function to inhibit the entry of potentially dangerous organisms and chemicals (Zareie et al., 2006). The frequently used bacterial genera are Lactobacillus species, Bifidobacterium species, and Streptococcus species (Butel, 2014).

On the other hand, prebiotics are substances that are not digestible, but have beneficial effects on the host by selectively stimulating the growth and activity of one or several bacteria, thereby improving host health. It is food ingredients for microorganisms and can promote the growth of beneficial bacteria in the human body (Florowska et al., 2016). Most commercially available prebiotics are oligosaccharides, such as isomaltooligosaccharide (IMO), fructooligosaccharide (FOS), and galactooligosaccharide (GOS), most of which are digested by Bifidobacterium spp. and promote the growth of intestinal bacteria. As prebiotics must meet requirements, when consumed, it is not decomposed and absorbed in the upper part of the digestive tract, but fermented by specific colonic microorganisms, exerting its facilitative function on the growth and metabolic activity of intestinal bacteria (Slavin, 2013).

Probiotics regulate intestinal flora and maintain the balance of intestinal microflora. Probiotics can produce SCFAs, antimicrobial peptides, and bacteriocins to inhibit the growth of pathogenic microorganisms, to accelerate the synthesis of the intestinal epithelium, and to protect gastrointestinal mucosa (Fooks & Gibson, 2002). Some probiotics also secrete different non-bacteriocin components to reduce the adherence of pathogenic microorganisms to mucosal surfaces and epithelial cells or to directly replace pathogenic microorganisms that bind to the
surface of cells or mucosal sites. For example, the antimicrobial substance reuterin produced by L. reuteri can inhibit the multiplication of pathogenic intestinal bacteria (Walter et al., 2011).

Probiotics stimulate intestinal mucosa to produce anti-inflammatory cytokines, enhance intestinal immunity, and thus induce immune enhancement or immunosuppression (Foligne et al., 2013). Through interactions with specific microorganisms or intestinal epithelial cells, probiotics induce T cells and B cells to produce specific signaling molecules like cytokines and chemokines. Regulating cell signaling and immune cell activity can inhibit the activities of inflammatory factors such as TLR4, TNF-α, and NF-κB and enhance the immune effect of the intestinal mucosa (Hardy et al., 2013). Bifidobacterium strains can reduce the intestinal inflammatory response by lowering the expression of TLR2 and TLR4 in the colon, and reducing the contact and uptake between intestinal microflora abnormal antigen and TLR (Bai & Ouyang, 2006). Evrard et al (2011) reported that the IL-23 member of IL-12 induced by Lactobacillus rhamnosus Lcr35 can accelerate the differentiation of Th17 cells and participate in the inflammatory response (Evrard et al., 2011).

The physiological functions of prebiotics play a major role in the gut, cardiovascular system, and skeleton. Prebiotics can effectively improve the composition and activity of intestinal microorganisms, such as bifidobacteria and lactic acid bacteria, promoting the proliferation of intestinal microbiota, optimizing the intestinal microbiota, and inhibiting the reproduction of spoilage bacteria (Yoo & Kim, 2016). Gaggia, Mattarelli, & Biavati (2010) have found that inulin can significantly increase intestinal probiotics, and improve the viability of probiotics, thus regulating the composition and function of intestinal microbial communities, as well as significantly reducing the number of E. coli (Gaggia, Mattarelli, & Biavati, 2010). Some prebiotics are non-digestible fiber compounds, which can stimulate intestinal peristalsis.
Therefore, they have a two-way regulation function, which can relieve diarrhea and prevent constipation. At the same time, it can enhance immunity and prevent inflammatory diseases of the GI tract (Hardy et al., 2013). Fukuda & Ohno (2014) found that after injecting a certain amount of inulin into mice, intestinal micro-ecology is improved and the secretion of lymph antibody is increased, which helps improve host immunity (Fukuda & Ohno, 2014).

1.5.1 *Lactobacillus pentosus* strain S-PT84

*Lactobacillus* spp. are frequently used in fermented foods and possess potential physiological properties including anti-inflammatory activities and effects on lipid absorption and metabolism (Zhou et al., 2013). *L. pentosus* strain S-PT84 is a lactic acid bacterium of plant origin isolated from Kyoto pickles (shibazuke), which is capable of enhancing the immune system to prevent infections and allergies (Izumo et al., 2010). It has been demonstrated that *L. pentosus* strain S-PT84 decreased the number of *Salmonella typhimurium* in mice feces, which was effective for maintenance of host health or for improvement of several symptoms during pathogen infection (Izumo et al., 2011). Another study discovered that *L. pentosus* strain S-PT84 could stimulate the production of IL-12 or IFN-γ, which is critical for innate and adaptive immunity (Izumo et al., 2011). These results account for the immune-modulatory effects of the *L. pentosus* strain S-PT84.

1.5.2 Isomaltodextrin (IMD)

Isomaltodextrin (IMD), a highly branched α-glucan, is enzymatically produced from maltodextrin by bacterial strain PP710 (Nishimura, Tanabe, & Yamamoto, 2016). An α-glucan is a polysaccharide of D-glucose linked with glycosidic bonds such as α-1, 4- and α-1, 6 glucans, mostly digestive and absorbable. The structure of IMD is like other dextrins and maltodextrins. IMD is comprised of α-glucan containing approximately “17% of α-1 glucosidic linkages, 3%
of $\alpha$-1, 3 glucosidic linkages, 19% of $\alpha$-1, 4 glucosidic linkages, 49% of $\alpha$-1, 6 glucosidic linkages, 7% of $\alpha$-1, 3, 6 glucosidic linkages, and 5% of $\alpha$-1, 4, 6 glucosidic linkages” (Figure 4) (GRAS Notice 610: Isomaltodextrin - FDA). Because of the degree and arrangement of linkages and bonds, IMD cannot be digested by the enzymes from the intestine and may have the potential for use as prebiotics. One study showed that IMD could significantly reduce the expression of pro-inflammatory cytokines and decrease inflammation in mouse colonic tissue, suggesting it might have anti-inflammatory efforts (Majumder et al., 2017). IMD, as the proliferation factor of *Bifidobacterium*, helps this organism multiply rapidly. Another study found IMD lowers the Firmicutes/Bacteroidetes ratio in the intestine and increases the level of *Bifidobacterium* (Nishimura, Tanabe, & Yamamoto, 2016). Thus, the anti-inflammatory properties of IMD may be achieved by altering intestinal microflora.
Figure 3. The structure of IMD. IMD is comprised of α-glucan containing approximately 17% of α-1 glucosidic linkages, 3% of α-1, 3 glucosidic linkages, 19% of α-1, 4 glucosidic linkages, 49% of α-1, 6 glucosidic linkages, 7% of α-1, 3, 6 glucosidic linkages, and 5% of α-1, 4, 6 glucosidic linkages (Adapted from FDA GRAS).
2. Hypothesis and Objectives

2.1 Hypothesis

The main hypothesis of this project is that LPS-induced chronic low-grade inflammation will change the composition and quantity of gut microbiota in mice. In addition, probiotics and prebiotics can improve gut microbiota and suppress gut dysbiosis.

2.2 Objectives

The objectives of this project are 1) to determine how LPS-induced low-grade inflammation affects the composition and quantity of gut microbiota in C57BL/6 mouse model, and 2) to evaluate *L. pentosus* strain S-PT84 and IMD for their ability to change and regulate the gut microbiota in C57BL/6 mouse model.

3. Materials and Methods

3.1 Reagents and Materials

Lipopolysaccharide (LPS) from *E. coli* 0111:B4 was used to induce low-grade inflammation in mice (Sigma, Oakville, Canada). Probiotic *L. pentosus S-PT 84* was kindly acquired from fermented Japanese pickle-*Shibazuke* (Kyoto, Japan) and prebiotics, isomaltodextrin (IMD) were generously provided by Hayashibara Co Ltd. (Okayama, Japan). QIAamp DNA Stool Mini Kit was used to extract genomic DNA of fecal bacteria (Qiagen, Mississauga, ON, Canada). Primers were synthesized by the University of Guelph Laboratory Services Molecular Biology Section (Guelph, Ontario, Canada). SYBR® Green PCR Master Mix and SYBR® Green RT-PCR Reagents Kit were used to perform PCR amplification (Thermo Fisher, USA) on a MyiQ™ Single Color Real-Time PCR Detection System (Bio-Rad Laboratories, Mississauga, ON). Hewlett Packard 5890 Series II system equipped with J & W
columns (CP-Wax 52 CB 30 × 0.53) (Agilent Technologies, Amstelveen, The Netherlands) was used for gas chromatographic analysis of SCFAs. 2-Ethylbutyric acid, used as internal standard, was purchased from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany). All water used was purified using a Milli-Q® reagent water system (Millipore, Molsheim, France).

3.2 Animal Study

The study was approved by the University of Guelph Animal Care Committee (The Animal Utilization Protocol (AUP) number is 3502). A total of 144 C57BL/6 female mice (Charles River Laboratories, Inc., Montreal, Quebec) were divided into two animal trials: *L. pentosus* S-PT84-treated animal trail and IMD-treated animal trail. Each trial contained five groups (negative control (NC) group; positive control (PC) group; low-dose (TL) *L. pentosus* S-PT84 group or IMD group; medium-dose (TM) *L. pentosus* S-PT84 group or IMD group; high-dose (TH) *L. pentosus* S-PT84 group or IMD group) and vehicle group. Each group including 12 mice (four mice per cage) were fed with standard rodent diet and maintained in a controlled environment (12h light/dark cycle and light off at 7 pm).

The entire animal trial lasted 16 weeks. From week 1 to week 16, mice in NC group were fed with normal 14% protein diet and water; mice in each vehicle group were fed with 14% protein diet and water with 0.6 % *L. pentosus* S-PT84 or 5.0% IMD. During the four-week pre-treatment, mice in PC group were given normal 14% protein diet and water; the mice in *L. pentosus* S-PT84 treatment groups were given different dosages 0.006%, 0.06% and 0.6% (w/w) of single pellet and the mice in IMD treatment groups were given 1.0 %, 2.5%, and 5.0% (w/v) in drinking water for food intervention. After the four-week pre-treatment, LPS derived from non-pathogenic *E. coli* 0111:B4 was orally administrated at the dosage of 300 μg/kg body weight/day via drinking water to PC, *L. pentosus* S-PT84 treatment, and IMD treatment groups
to initiate low-grade inflammation (Table 3).
Table 3. The animal trials designed for a) probiotic *L. pentosus* S-PT84 and b) prebiotic IMD-treated mice. C57BL/6J mice in NC group received 14% rodent diet and autoclaved water from week 1 to week 16. Mice in PC group received 14% rodent diet for 16 weeks. a) In probiotic *L. pentosus* S-PT84 trial, mince in TL, TM, and TH group were given 14% rodent diet with 0.006 wt%, 0.06 wt%, or 0.6 wt% of *L. pentosus* S-PT84 respectively for 12 weeks. b) In prebiotic IMD trial, mince in TL, TM, and TH group were given 14% rodent diet with 1% (w/v), 2.5% (w/v), or 5% (w/v) of IMD respectively for 12 weeks. From week 5, LPS (300 μg/kg BW/day) was added to drinking water of PC, TL, TM, and TH in two animal trials to induce inflammation.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Pre-treatment (weeks 1-4)</th>
<th>Treatment (weeks 5-16)</th>
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<tbody>
<tr>
<td>Negative control (NC)</td>
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<td></td>
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<td>Positive control (PC)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>in water</td>
</tr>
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<td>Low dose treatment (TL)</td>
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<td>0.006% S-PT84</td>
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<td>Medium dose treatment (TM)</td>
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<tr>
<td>High dose treatment (TH)</td>
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</tr>
<tr>
<td></td>
<td>0.6% S-PT84</td>
<td>0.6% S-PT84</td>
</tr>
<tr>
<td>Vehicle control (TC)</td>
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<tr>
<td></td>
<td>0.6% S-PT84</td>
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<tr>
<td>Treatment Group</td>
<td>Pre-treatment (weeks 1-4)</td>
<td>Treatment (weeks 5-16)</td>
</tr>
<tr>
<td>-------------------------</td>
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<tr>
<td>Negative control (NC)</td>
<td>14% rodent diet + water</td>
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<tr>
<td>Positive control (PC)</td>
<td>14% rodent diet + water</td>
<td>14% rodent diet + LPS in water</td>
</tr>
<tr>
<td>Low dose treatment (TL)</td>
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<td>14% rodent diet + LPS in water 1% IMD</td>
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<tr>
<td>Medium dose treatment (TM)</td>
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<td>High dose treatment (TH)</td>
<td>14% rodent diet + water 5% IMD</td>
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<tr>
<td>Vehicle control (TC)</td>
<td>14% rodent diet + water 5% IMD</td>
<td></td>
</tr>
</tbody>
</table>
3.3 Real Time PCR

3.3.1 Feces Collection

Fecal samples from each group were collected every two weeks and then were stored at −80°C until DNA extraction.

3.3.2 DNA Extraction

Bacterial DNA was extracted from fecal samples using the Qiagen QIAamp DNA stool mini kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer’s “Isolation of DNA from Stool for Pathogen Detection” protocol. Briefly, 200 mg feces were mixed with buffer and InhibitEX to remove many compounds that could degrade DNA and inhibit downstream enzymatic reactions. After vortex and centrifugation at 10 000 x g for 1 min, the DNA in the supernatant was purified on QIAamp Mini spin columns. DNA bound to the QIAamp membrane by centrifugation and was eluted from the QIAamp Mini spin column in a low-salt buffer. The overall quality and quantification of total DNA were assessed using the NanoDrop™ 8000 Spectrophotometer (Thermo fisher, Wilmington, DE, USA) and 1.5 μL of each sample was used for each measurement. Samples were stored at −20 °C until further treatment. DNA concentrations were diluted to a final concentration of 12.5 ng/μL for PCR amplification. The quality of DNA was determined by calculating A260/A280 ratio and was considered pure between 1.8 and 2.2.

3.3.3 Primer Preparation

A set of 16S rRNA gene-targeted group-specific primers were designed for microbial identification, including *B. bifidum*, *L. casei*, *E. coli*, *C. difficile* and *B. fragilis*, using GenBank (http://www.ncbi.nlm.nih.gov/) and synthesized by the University of Guelph Laboratory Services Molecular Biology Section (Guelph, Ontario, Canada) (Table 4). 16S rRNA is a component of
the small subunit of prokaryotic ribosomes and is ubiquitous in all microbes. Also, the order of certain bases in the 16S rRNA molecule is very conservative and contains hypervariable regions that make it suitable for microbial identification (Kim et al., 2011).
<table>
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<th>Reserve primer (5'-3')</th>
<th>Product (bp)</th>
<th>Accession No.</th>
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</thead>
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<td>attaccgccccgtcctgcc</td>
<td>200</td>
<td>Walter, J., et al., 2006</td>
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</tbody>
</table>
3.3.4 Real-time PCR

Amplification of DNA was accomplished using real-time quantitative PCR. Real-time quantitative PCR was performed using SYBR® Green PCR Master Mix (Thermo Fisher) on MyiQ™ Single Color Real-Time PCR Detection System (Bio-Rad Laboratories) using the following conditions: denaturation at 95°C for 15 min followed by 60 cycles of denaturation at 95°C for 5 secs, annealing at 55°C for 15 sec, extension at 72°C for 30 sec, and a final 80 cycles of extension at 55°C for 30 sec. Samples were cooled down at 4°C, and then further analysis was carried out. For each reaction, 2 µl of template DNA (12.5 ng/µL) was mixed with 1 µl of forward and reverse primers (5 pmol/l) of target bacteria, 10 µl of SYBR® Green Mix, and 6 µl of sterile water to make a total reaction volume of 20 µl. The melting curve analysis was implemented after each assay to distinguish a specific PCR product from non-specific amplification. After qPCR assay, the Ct values were determined. A standard curve was not performed because this is a relative quantification approach. Results were expressed as fold changes relative to the negative control group as control and relative microbial population expression was analyzed using the 2^\(-\Delta\Delta Ct\) methods (Livak & Schmittgen, 2001).

3.4 Analysis of Fatty Acids by Gas Chromatography (GC)

Gas chromatography (GC) was used to determine fecal volatile fatty acid concentrations. Mice fecal samples were prepared and analyzed as recently described (Zarepoor et al., 2014). Briefly, 300 mg of freeze-dried samples were collected and freeze dried for two days. Three-milliliter MilliQ water was added and mixed by vortex to get 10% (w/v) homogenous fecal solution. Five mol/L HCl was then added to adjust pH to 2-3 and kept for 10 min at room temperature. The solution was vortexed and centrifuged at 10 000 x g for 10 min. 50 µl 2-
ethylbutyric acid (Aldrich, #109959) was added to the supernatant as an internal standard. The supernatant was filtered through a 0.2 μm PVDF syringe filter and injected (1μl) into the gas chromatograph. Chromatographic analysis was carried out using Hewlett Packard 5890 Series II system equipped with J & W columns (CP-Wax 52 CB 30 × 0.53) (Agilent Technologies, Amstelveen, The Netherlands). Injector and detector temperatures were maintained at 240°C and 280°C, respectively. After an initial period of 1 min at 75°C, oven temperature was increased to 180°C at a rate of 6°C/min and then increased by 10°C/min and held at 230°C for 6 min. The final oven temperature was increased by 2°C/min and kept at 240°C for 5 min. The peaks were identified by comparison of their retention times with acetate, propionate, and butyrate gas chromatography standards (Sigma-Aldrich, Oakville, ON, Canada).

3.5 Statistical Analysis

Data are expressed as means ± SEM. Statistical analysis was performed with SAS 9.2 (SAS Institute, Inc., Cary, NC, USA) using two-way analysis of variance (ANOVA) and Duncan’s multiple range tests. A p-value < 0.05 was considered significant.

4. Results

In the study, real-time RT-PCR was performed to determine the quantity change of five bacteria that were involved in regulating and maintaining the balance of gut intestine in mice with low-grade inflammation. Fecal samples (n=6) from the five groups of mice were used for RT-PCR and GC analysis. A total of 105 mice fecal samples were included in the final analysis, 60 fecal samples from probiotic-treatment mice and 45 fecal samples from prebiotic-treatment mice.

Primer Specificity and Data Calculation
In order to check primer specificity to the input PCR template, primers for target bacteria were designed and applied in RT-PCR amplification. The melting curves shown in Figure 5 reveal the specific primer for each bacterium.

qRT-PCR uses fluorescence signals to monitor the amount of change of the product in the PCR amplification in real time, which can lead to a precise quantitative analysis of the initial template. Figure 6 shows the PCR amplification graph. At the early stage of PCR, the primers searched for complementary sequences on single stranded DNA templates and combined with specific sites. With the proper concentration of DNA templates and primer, the amplification reaction was carried out smoothly, and the product grew exponentially. The later stage was the platform stage where the excessive loss of DNA polymerase or the product led to slow reaction.

In the RT-PCR analysis, relative microbial population expression was calculated using the $2^{-\Delta \Delta Ct}$ method as described by Livak and Schmittgen (2001). $2^{-\Delta \Delta Ct}$ is a simple method to analyze the relative changes of gene expression in a real-time quantitative PCR experiment. In this experiment, Eubacteria gene was used as reference gene. Results were presented as fold change of microbial population expression relative to the negative control group. The $C_t$ values could be obtained from real-time PCR instrumentation, which represented the number of cycles experienced when the fluorescence signal reached the set field value in each reaction (Figure 6). The data were calculated as following

$\Delta Ct = Ct$ (target) — $Ct$ (all bacteria)

$\Delta \Delta Ct = \Delta Ct$ (target) — $\Delta Ct$ (average)

Fold change of microbial population expression $= 2^{\Delta \Delta Ct}$
Figure 5. PCR quantification with melting curves implemented for primer specification verification. (a, *B. bifidum*; b, *L. casei*; c, *E. coli*; d, *C. difficile*; e, *B. fragilis*; and f, Eubacteria)
Figure 6. PCR amplification/cycle graph of *L. casei* and *C. difficile* in week 4 (a, *L. casei* and b, *C. difficile*). The Ct value used to calculate the relative microbial population expression is the number of cycles when the fluorescence signal reaches the set field value in each reaction.
The LPS-induced low-grade inflammation in the C57BL/6J mouse model has been established (Majumder et al., 2017). Administration of 300 μg/kg BW/day LPS in C57BL/6J mice for 16 weeks resulted in intestinal inflammation and systemic low-grade chronic inflammation with increased concentrations of pro-inflammatory mediators in the plasma, such as TNF-α and MCP-1, as well as the increased concentrations of endotoxin. Intestinal inflammation and systemic low-grade chronic inflammation reduced the production of anti-inflammatory mediators, such as adiponectin. In addition, the concentration of D-mannitol in plasma is considered to be an index used to measure intestinal permeability. Mice in the PC group had a higher concentration of D-mannitol in plasma, indicating the PC group had increased intestinal permeability. All these results have demonstrated that LPS induces low-grade chronic inflammation in the C57BL/6J mouse model.

4.1 Effect of Probiotic L. pentosus S-PT 84 on Microbiota Population

In the present study, the relative populations of five bacterial species were analyzed using qRT-PCR to evaluate the effects of L. pentosus S-PT84 on the composition of the intestinal microbiota. Probiotic L. pentosus S-PT84 helped increase the relative population of B. bifidum, L. casei, and B. fragilis, and decrease the relative population of E. coli and C. difficile.

As shown in Figure 7, comparing to the NC group, the PC group of mice showed a significant decrease in the relative population of B. bifidum, L. casei, and B. fragilis, and a significant increase in the relative population of E. coli and C. difficile. Comparing to the PC group, mice in the TL, TM, and TH groups showed a significant increase in the relative population of B. bifidum, L. casei, and B. fragilis, and a significant decrease in the relative population of E. coli and C. difficile. A two-way ANOVA was used for statistical analysis.

Duncan’s multiple range tests were used for the analysis of each group at different weeks.
As shown in Figure 7, compared to week 0, PC group mice showed a significant decrease in the relative population of *B. bifidum*, *L. casei*, and *B. fragilis*, but a significant increase in *E. coli* and *C. difficile* at weeks 12 and 16. Mice in the TL and TM groups showed no significant difference in the relative population of *B. bifidum*, *L. casei*, *B. fragilis*, *E. coli*, and *C. difficile* at weeks 12 and 16 when compared to week 0. In the TH group, there was a significant increase in the relative population of *B. bifidum*, *L. casei*, and *B. fragilis*, but a significant decrease in the relative population of *E. coli* and *C. difficile* at weeks 12 and 16 when compared to week 0.

In order to clearly compare the change in the relative population of each of the five bacteria, the percentage of each was calculated. When comparing weeks 0 and 4 (Figure 8a and b), the results revealed a strong effect of *L. pentosus* S-PT84 in which the relative populations of *B. bifidum* and *L. casei* increased, while *E. coli* and *C. difficile* decreased in the *L. pentosus* S-PT84 treated groups. More specifically, the percentage of *B. bifidum* and *L. casei* increased from 20% to 31% and 20% to 26% respectively in the TH group, while the percentage of *E. coli* and *C. difficile* decreased from 20% to 11%, and from 20% to 7.5% respectively. In addition, the relative population of *B. fragilis* increased slightly, from 20% to 24% in the TH group (Figure 7e).

The probiotic *L. pentosus* S-PT84 showed a strong protective effect on the composition and quantity of bacterial communities in mice exposed to LPS. When comparing week 4 and week 16 (Figure 8b and c), the proportion of *B. bifidum* and *L. casei* in the PC group decreased from 20% to 7% and 20% to 10% respectively, while the percentage of *E. coli* and *C. difficile* increased from 20% to 35% and 20% to 34% respectively. In contrast, compared to the PC group, the relative population of *B. bifidum*, *L. casei*, and *B. fragilis* increased in the *L. pentosus* S-PT84 treatment groups, while the relative population of *E. coli* and *C. difficile* were suppressed.
Among three *L. pentosus* S-PT84 treatment groups, the TH group showed the most prominent effect on the improvement of gut microbiota composition. After *L. pentosus* S-PT84 treatment, *B. bifidum* decreased by 13% in the PC group, while the percentage of this organism increased to 31% in the TH group. More than one-third of the bacteria population was *E. coli* in the PC group, but this number dropped to 10% in the TH group. However, even if the LPS administration continued for 12 weeks, the beneficial bacteria returned to predominant levels at week 16 (Figure 8c). Therefore, it can be deduced that the supplement with probiotic *L. pentosus* S-PT84 prevents the decrease of beneficial microbiota.
Figure 7. Relative population expression levels of *B. bifidum*, *L. casei*, *E. coli*, *C. difficile*, and *B. fragilis* in probiotic *L. pentosus* S-PT84-treated mice. The significant differences with treatment time are shown by asterisk, *p* < 0.05, compared with the PC group. The different letters showed the significant differences under treatment (*p* < 0.05). Data are represented as mean ± SEM of *B. bifidum*, *L. casei*, *E. coli*, *C. difficile*, and *B. fragilis* (n=6). Note: The samples from vehicle control (VC) group are not used in the analysis.
Figure 8. Effect of probiotic \textit{L. pentosus} S-PT84 on the percentage change of each bacterium compared to the total bacterial population in LPS-induced low-grade inflammatory mice. NC, negative control (untreated mice); PC, positive control (mice treated with LPS); TL, treatment low (mice treated with 0.006% S-PT84 and LPS); TM, treatment medium (mice treated with 0.06% S-PT84 and LPS); TH, treatment high (mice treated with 0.6% S-PT84 and LPS). The stacked bar graph is presented as the relative percentage of \textit{Bifidobacterium bifidum}, \textit{Lactobacillus casei}, \textit{Escherichia coli}, \textit{Clostridium difficile}, and \textit{Bacteroides fragilis} on week 0, 4, and 16 (n=6). Note: The samples from vehicle control (VC) group are not used in the analysis.
4.2 Effect of Prebiotic IMD on Microbiota Population

As discussed previously, the prebiotic IMD acts as food that can induce the growth of beneficial bacteria, such as *B. bifidum* and *L. casei*. The effect of the prebiotic IMD on the relative population of five bacterial species was analyzed by qRT-PCR.

As shown in Figure 9, compared to the NC group, the PC group mice showed a significant decrease in the relative population of *B. bifidum*, *L. casei*, and *B. fragilis*, but a significant increase in the relative population of *E. coli* and *C. difficile*. Compared to the PC group, mice in the TL, TM, and TH groups showed a significant increase in the relative population of *B. bifidum*, *L. casei*, and *B. fragilis*, and a significant decrease in the relative population of *E. coli* and *C. difficile*. A two-way ANOVA was used for statistical analysis.

Each group at different weeks was also analyzed using Duncan’s multiple range tests. As shown in Figure 9, the PC group showed a significant decrease in the relative population of *B. bifidum* and *L. casei*, but a significant increase of *E. coli* and *C. difficile* at weeks 12 and 16 when compared to week 0. In the TL group, there was no significant difference in the relative population of *B. bifidum*, *L. casei*, *B. fragilis*, and *E. coli* at weeks 4, 12, and 16 when compared to week 0. The TM and TH groups showed a significant increase in the relative population of *B. bifidum* and *L. casei*, but a significant decrease in the relative population of *E. coli* and *C. difficile* at weeks 4, 12, and 16 when compared to week 0. The relative population of *B. fragilis* increased in the TL, TM, and TH groups but decreased in the PC group at weeks 4, 12 and 16 when compared to week 0, but the difference was not statistically significant.

To determine the effects of the prebiotic IMD on gut microbiota, the proportion of each of the five bacteria was calculated (Figure 10). The prebiotic IMD had a strong impact on gut
microbiota that the percentage of *B. bifidum*, *L. casei*, and *B. fragilis* in IMD treatment groups increased significantly, while *E. coli* and *C. difficile* reduced from week 0 to week 4 (Figure 10 a and b). Specifically, the relative population of *B. bifidum* and *L. casei* increased from 19% to 28% and 19% to 27% respectively in the TM group, but *E. coli* and *C. difficile* decreased from 21% to 10% and 21% to 13% respectively. The ability of IMD to alter intestinal microflora varied depending on different doses of IMD. The percentage of beneficial bacteria in the TM group was 56%, which was similar to that in the TH group. Thus the TM group revealed the most optimal effect when compared to the TL and TH groups.

In addition, the prebiotic IMD improved the composition and quantity of bacterial communities in mice with LPS treatment (Figure 10 b and c). As shown in Figure 10 b-c, LPS inhibited the growth of *B. bifidum*, *L. casei*, and *B. fragilis*, but promoted the growth of *E. coli* and *C. difficile* in the PC group from week 4 to week 16. In the PC group, the proportion of *B. bifidum* and *L. casei* decreased from 21% to 9% and 20% to 9% respectively, while the percentage of *E. coli* and *C. difficile* increased from 20% to 34% and 20% to 35% respectively. Compared to the PC group, the IMD treatment groups maintained the predominant level of *B. bifidum*, *L. casei*, and *B. fragilis* with a small percentage of *E. coli* and *C. difficile* (Figure 10c). Twelve-weeks of LPS treatment did not reduce the beneficial bacteria. To some extent, the prebiotic IMD could alleviate the damage of LPS to the gut intestine and optimize the intestinal microflora composition.
Figure 9. Relative population expression levels of *B. bifidum*, *L. casei*, *E. coli*, *C. difficile*, and *B. fragilis* in prebiotic IMD-treated mice. The significant differences with treatment time are shown by asterisk, *p* < 0.05, compared with the PC group. The different letters showed the significant differences under treatment (*p* < 0.05). Data are represented as mean ± SEM of *B. bifidum*, *L. casei*, *E. coli*, *C. difficile*, and *B. fragilis* (n=6). Note: The samples from vehicle control (VC) group are not used in the analysis.
Figure 10. Effect of prebiotic IMD on the percentage change of each bacterium compared to the total bacteria population in LPS-induced low-grade inflammatory mice. NC, negative control (untreated mice); PC, positive control (mice treated with LPS); TL, treatment low (mice treated with 1% IMD and LPS); TM, treatment medium (mice treated with 2.5% IMD and LPS); TH, treatment high (mice treated with 5% IMD and LPS). The stacked bar graph is presented as the relative percentage of *B. bifidum*, *L. casei*, *E. coli*, *C. difficile*, and *B. fragilis* on week 0, 4, and 16 (n=6). Note: The samples from vehicle control (VC) group are not used in the analysis.
4.3 The Levels of SCFAs in Mice Feces Analysis

As discussed previously, the *L. pentosus* S-PT84 treated high group and the IMD treated medium group had the best effects on gut microbiota composition. C57BL/6 mice feces from NC, PC, the *L. pentosus* S-PT84 treated high group and the MD treated medium group at weeks 0, 4, and 16 were used to determine the concentrations of SCFAs by gas chromatography analysis. There were three main SCFAs detected: acetic acid, propionic acid, and butyric acid.

Tables 6 and 7 show the concentrations of acetic acid, propionic acid, and butyric acid in the NC, the PC, the *L. pentosus* S-PT84 treated high group, and the MD treated medium group at weeks 0, 4, and 16. Butyric acid had the highest concentration, followed by acetic acid and propionic acid.

As shown in Table 5, compared to the NC group, there was no significant difference in the concentration of acetic acid, propionic acid, and butyric acid in the PC group. The *L. pentosus* S-PT84 treated high group showed a significant increase of acetic acid (4.03±0.92 μg/ml at week 4 and 3.87±1.13 μg/ml at week 16, *p* <0.05) and butyric acid (18.30±2.29 μg/ml at week 4 and 14.43±2.85 μg/ml at week 16, *p* <0.05) compared to the PC group (2.44±0.45 μg/ml at week 4 and 2.22±0.30 at week 16, *p* <0.05). A Two-way ANOVA test was performed for statistical analysis.

As shown in Table 5, the PC group showed no significant difference in the concentration of acetic acid and propionic acid, but a significant decrease of butyric acid concentration (8.40±3.40 μg/ml at week 4 and 9.10±2.25 μg/ml at week 16, *p* <0.05) compared to week 0 (13.57±0.15 μg/ml). The *L. pentosus* S-PT84 treated high group showed no significant difference in the concentration of acetic acid and propionic acid, but a significant decrease of butyric acid.
concentration (18.30±2.29 μg/ml at week 4 and 14.43±2.85 μg/ml at week 16, \(p < 0.05\)) compared to week 0 (13.87±2.80 μg/ml). Duncan’s multiple range tests were performed for statistical analysis.

As shown in Table 6, compared to the NC group (3.73±0.74 μg/ml and 12.13±0.04 μg/ml), there was a significant decrease in the concentration of acetic acid (1.63±0.17 μg/ml) and butyric acid (5.33±1.32 μg/ml) in the PC group. The IMD treated medium group showed a significant increase of acetic acid (4.39±1.55 μg/ml) and butyric acid (15.10±1.39 μg/ml) compared to the PC group (1.63±0.17 μg/ml and 5.33±1.32 μg/ml). A Two-way ANOVA test was performed for statistical analysis.

As shown in Table 6, the PC group showed no significant difference in the concentration of acetic acid and propionic acid, but a significant decrease of butyric acid concentration (5.33±1.32 μg/ml) at week 16 compared to week 0 (13.57±0.15 μg/ml). The IMD treated medium group showed no significant difference in the concentration of propionic acid and butyric acid, but a significant decrease of acetic acid concentration (5.56±0.60 μg/ml at week 4 and 4.39±1.55 at μg/ml week 16, \(p < 0.05\)) compared to week 0 (2.57±0.71 μg/ml). Duncan’s multiple range tests were performed for statistical analysis.
Figure 11. Gas chromatographic profiles of SCFAs in mice feces. a) Probiotic-treated mice feces in week 0. b) Probiotic-treated mice feces in week 16. c) Prebiotic-treated mice feces in week 0. d) Prebiotic-treated mice feces in week 16. Peak identification: acetic acid, propionic acid, and butyric acid.
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<th>SCFA/Concentration (μg/ml)</th>
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<th>Butyric Acid</th>
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<td>NC</td>
<td>2.81±0.79 a</td>
<td>0.49±0.18 a</td>
<td>14.63±2.47 a</td>
</tr>
<tr>
<td>PC</td>
<td>3.17±0.37 a</td>
<td>0.73±0.03 a</td>
<td>13.57±0.15 a</td>
</tr>
<tr>
<td>TH</td>
<td>2.57±0.71 a</td>
<td>0.62±0.18 a</td>
<td>13.87±2.80 a</td>
</tr>
<tr>
<td>Week 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>3.95±0.64 a</td>
<td>0.64±0.07 a</td>
<td>14.50±0.95 a</td>
</tr>
<tr>
<td>PC</td>
<td>2.44±0.45 a</td>
<td>0.74±0.43 a</td>
<td>8.40±3.40 b</td>
</tr>
<tr>
<td>TH</td>
<td>4.03±0.92 a*</td>
<td>0.32±0.08 a</td>
<td>18.30±2.29 b*</td>
</tr>
<tr>
<td>Week 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>3.37±0.90 a</td>
<td>0.50±0.08 a</td>
<td>13.53±0.64 a</td>
</tr>
<tr>
<td>PC</td>
<td>2.22±0.30 a</td>
<td>0.54±0.13 a</td>
<td>9.10±2.25 b</td>
</tr>
<tr>
<td>TH</td>
<td>3.87±1.13 a*</td>
<td>0.41±0.09 a</td>
<td>14.43±2.85 b*</td>
</tr>
</tbody>
</table>

Values are expressed in mean± standard deviation (SD) of acetic acid, propionic acid, and butyric acid. NC, negative control (untreated mice); PC, positive control (mice treated with LPS); TH, treatment high (mice treated with 0.6% S-PT84 and LPS). The significant differences are shown by asterisk, * p < 0.05, compared with positive control group. The different letters showed the significant differences under treatment (p < 0.05). Data are represented as mean ± SEM of B. bifidum, L. casei, E. coli, C. difficile, and B. fragilis (n=6).
Table 6. Concentrations of acetic, propionic, and butyric acids in fecal samples from IMD-treated mouse trial.

<table>
<thead>
<tr>
<th>SCFA/Concentration (μg/ml)</th>
<th>Acetic Acid</th>
<th>Propionic Acid</th>
<th>Butyric Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>2.81±0.79 a</td>
<td>0.49±0.18 a</td>
<td>14.63±2.47 a</td>
</tr>
<tr>
<td>PC</td>
<td>3.17±0.37 a</td>
<td>0.73±0.03 a</td>
<td>13.57±0.15 a</td>
</tr>
<tr>
<td>TM</td>
<td>2.57±0.71 a</td>
<td>0.62±0.18 a</td>
<td>13.87±2.80 a</td>
</tr>
<tr>
<td>Week 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
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<td>0.71±0.02 a</td>
<td>11.80±0.44 a</td>
</tr>
<tr>
<td>PC</td>
<td>2.39±0.45 a</td>
<td>0.31±0.06 a</td>
<td>12.31±1.31 a</td>
</tr>
<tr>
<td>TM</td>
<td>5.56±0.60 b*</td>
<td>0.81±0.10 a</td>
<td>16.77±2.80 a</td>
</tr>
<tr>
<td>Week 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>3.73±0.74 a*</td>
<td>0.55±0.06 a</td>
<td>12.13±0.04 a*</td>
</tr>
<tr>
<td>PC</td>
<td>1.63±0.17 b</td>
<td>0.74±0.06 a</td>
<td>5.33±1.32 b</td>
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<tr>
<td>TM</td>
<td>4.39±1.55 b*</td>
<td>0.59±0.13 a</td>
<td>15.10±1.39 a*</td>
</tr>
</tbody>
</table>

Values are expressed in mean± standard deviation (SD) of acetic acid, propionic acid, and butyric acid. NC, negative control (untreated mice); PC, positive control (mice treated with LPS); TM, treatment medium (mice treated with 5.0% IMD and LPS). The significant differences are shown by asterisk, * p < 0.05, compared with positive control group. The different letters showed the significant differences under treatment (p < 0.05). Data are represented as mean ± SEM of B. bifidum, L. casei, E. coli, C. difficile, and B. fragilis (n=6).
5. Discussion

5.1 Administration of LPS Enhances Disturbance of Intestinal Microflora and Low-Grade Inflammation

In the present study, the comparative analysis of results from the NC, PC and *L. pentosus* S-PT84-treated groups, and IMD-treated groups has shown a significant difference in the intestinal microbiota. In the PC group, LPS changed the balance of the intestinal microflora composition and reduced the number of beneficial bacteria. The results, on the one hand, might be related to the absence of probiotics or prebiotics intake in the PC group mice at the early stage of the study. On the other hand, the tight junctions of mice gut damaged by the low level of LPS administration increased intestinal permeability and caused gut microbiota translocation. The leaky gut promoted bacterial LPS translocation into the bloodstream and caused endotoxemia, resulting in low level of chronic inflammation (Moreira et al., 2012).

Increased gut permeability induced by LPS is associated with the changes of TJ proteins in intestinal epithelial cells (IECs), which affects the changes in gut microbiota composition (Moreno-Indias et al., 2014). Zonula occludens-1 (ZO-1) is basic structure and composition of tight junction and plays an important role in maintaining the integrity of tight junction structure. Under LPS stimulation, the expression of the ZO-1 protein is modified, the cytotoxic effect of intestinal epithelial cells is increased significantly, but the viability of cells is significantly decreased, which leads to an increase in cell permeability and apoptosis (Xiao et al., 2013). Moreover, translocation of intestinal bacteria and endotoxin could be observed after intestinal mucosal injury induced by LPS. It is worth noting that bacterial translocation is usually associated with the imbalance of intestinal microflora where the Gram-negative to Gram-positive ratio increases (Hoerr et al., 2012). With the decrease in the number of *Bifidobacterium* and
lactobacillus, indigenous microflora such as *E. coli*, *Staphylococcus*, and *Enterococcus faecalis* or opportunistic pathogens come into prominence. At the same time, these pathogenic bacteria cross the impaired intestinal epithelium into the organs and blood circulation, leading to endogenous infection and the formation of bacteremia or sepsis (Hentges, 2012).

In addition, intestinal-derived LPS reduce the production of glucagon like peptide 2 (GLP2) intestinal neuroendocrine L cells, thereby weakening the integrity of the colonic epithelium and enhancing inflammation (Greiner & Backhed, 2016). GLP2 is a specific gut peptide participating in the regulation of epithelial cell proliferation and gut barrier integrity. Cani et al (2012) have demonstrated that increased production of endogenous GLP-2 contributed to an improvement of the mucosal barrier function, resulting in decreased plasma LPS concentrations and improved tight junctions and therefore relieved inflammatory stresses (Cani et al., 2012). Thus, when redundant LPS crosses the gastrointestinal mucosa into the circulatory system through high intestinal permeability or absorption by enterocytes during chylomicron secretion, elevated LPS levels in blood plasma acts as a major inducer of the inflammatory response and contributes to endotoxemia and metabolic diseases (Boulange et al., 2016).

Furthermore, LPS-mediated activation of TLR4 is involved in the regulation of inflammatory processes associated with the onset of obesity and insulin resistance. LPS recognized by TLR4 up regulate the expression of proatherogenic LDL receptor, very low-density lipoprotein (VLDL) receptor, and adiponectin receptor 2 (AdipoR2) in macrophages (Vijay-Kumar et al., 2010). In this case, lipid uptake by macrophages can be increased, which facilitates their conversion to foam cells and enhances the risk of inflammation (Galkina & Ley, 2009). This result, in accordance with the many studies, has confirmed that LPS, as one of the
triggering factors, significantly contributes to the development of metabolic diseases and the cascade of inflammation (Calder et al., 2011; Boulange et al., 2016).

5.2 The Impact of Probiotic L. pentosus S-PT84 and Prebiotic IMD on Microbiota Population Alteration

The inflammatory effect of LPS administration on mice model was demonstrated. The symptoms of the gut-disturbed microflora and low-grade inflammation induced by LPS administration were alleviated when mice were treated with probiotic L. pentosus S-PT84 or prebiotic IMD. Since probiotics and prebiotics help regulate the intestinal flora to improve the symptoms of low-grade inflammation, which makes them as new prevention and treatment of metabolic diseases caused by intestinal dysbacteriosis and low-grade inflammation. At present, prebiotic IMD has not been widely used in the food industry like probiotic, but its potential benefits and anti-inflammatory mechanisms have been clarified by many studies (Heilbronn & Campbell, 2008).

Probiotics and prebiotics play a significant restoration role by modulating the composition of gut microflora (Lee, 2013). Their beneficial effect is also involved in regulating the diversity of gut microflora. In general, probiotics are made up of lactic acid bacteria and Bifidobacterium, which directly provide a probiotic source. Prebiotics containing Bifidus factors can effectively increase the content of Bifidobacterium in the intestinal tract and stimulate metabolic function, thereby reducing the impact of LPS-induced metabolic disorders (Femia et al., 2002; Cani et al., 2007). Many studies have demonstrated probiotics and prebiotics can lower the Firmicutes/Bacteroidetes ratio in the intestine and increase the level of bifidobacteria and Lactobacillus (Nishimura, Tanabe, & Yamamoto, 2016).
The mechanism of beneficial bacteria competing with pathogens for intestinal mucosal surface binding sites and then colonizing on the intestinal tract may be an effective way by which probiotics and prebiotics can control inflammatory condition in mice (Collado, Meriluoto, & Salminen, 2007). After entering the intestinal tract, probiotics first contact the surface of the intestinal membrane and bind to the adhesion receptor of the mucosal surface. This adhesion, on the one hand, forms a biological barrier by competing for site occupancy to prevent the pathogen from binding to the intestinal mucosal receptor. On the other hand, it also prolongs the time that probiotics affect the host gut immune system and the microbial flora, which is related to the efficiency of its physiological effects (Fijan, 2016). Candela et al (2008) have studied the role of *Lactobacillus* and *Bifidobacterium* strains on intestinal epithelial cells. The results have shown that *B. longum* Bar33, *B. lactis* Bar30, *Lacidophilus* Bar13, and *L. plantarum* Bar10 are effective in inhibiting the adhesion of *S. cholerasuis* serovar *Typhimurium* and *E. coli* to Caco-2 cells (Candela et al., 2008). Therefore, it is advantageous to establish a stable microbiome composed of a variety of native symbiotic microbial populations.

Besides competition from intestinal mucosal surface binding sites in the gut, probiotics and prebiotics can synthesize lactic acid and produce antibacterial substances to regulate microbial composition in the gut (De Keersmaecker et al., 2006). Studies have found that most probiotics can produce lactic acid through fermentation to reduce intestinal pH and inhibit the growth of microorganisms’ sensitive to the acidic environment, thus reducing pathogenic microorganisms in the intestines (Makras et al., 2006; Rios-Covian et al., 2016). Moreover, acidification of the colonic environment is not only harmful to some spoilage microorganisms such as *Bacteroides, Clostridium*, and *E. coli*, but also beneficial to the production of mucin, which improves the ecological environment of the intestinal mucosa and reduces the migration
and translocation of spoilage bacteria (van Loveren, Sanz, & Salminen, 2012). At the same time, some antibacterial substances such as hydrogen peroxide and bacteriocin can also be produced by *Lactobacillus acidophilic*, which inhibits the growth of harmful bacteria (Gillor et al., 2008). Accordingly, the use of probiotics and prebiotics may be involved in regulating the physiological environment of the gut to maintain microbial homeostasis. Indeed, some beneficial bacteria including obligate aerobic bacteria or facultative anaerobes consume large amounts of oxygen and reduce intestinal redox potential, which is suitable for the development of *Bifidobacterium* spp. and *Lactobacillus* spp. (Isolauri, Salminen, & Ouwehand, 2004).

Moreover, the significant growth of commensal bacteria is beneficial on the enhancement of mucosal barrier function and inhibition of bacterial translocation (Ohland & Macnaughton, 2010). In the current study, the significant decrease in beneficial microbiota following LPS treatment was mainly due to a drastic increase of gut permeability and induction of metabolic endotoxemia. Studies have suggested that the increased number of *Bifidobacterium* spp. is correlated with improved gut permeability and the improved gut barrier observed in mice is associated with lower plasma LPS levels and decreased circulating cytokines (Cani et al., 2008; Moreno-Indias et al., 2014). In other words, the number of *bifidobacteria* is negatively correlated with LPS levels and gut permeability. The mechanism by which *bifidobacteria* enhances gut permeability has been proved that *bifidobacteria* do not degrade intestinal mucus glycoproteins like *E. coli*, thus preventing gut permeability and bacterial translocation to promote healthy microvilli environment (Cani et al., 2008). While the role of probiotics and prebiotics as facilitating factor for beneficial bacteria growth has been explored, their role in enhancing mucosal barrier draws interest. Cani et al (2009) using obese mice model found that prebiotics modulation of intestinal microflora could increase villus height and crypt depth, resulting in an
increased thickness of the mucosa of the jejunum and colon (Cani et al., 2009). Another study has shown that probiotics Lactobacillus can improve the mucosal morphology of small intestine in mice and reduce the intestinal injury caused by *E. coli* LPS, which is helpful to optimize the intestinal environment and maintain intestinal microflora balance (Wang et al., 2016).

To further understand the intestinal barrier function regulated by probiotics and prebiotics in LPS-induced inflammatory mice, the implication of probiotics and prebiotics in intracellular signaling pathways needs to be studied. In the intestinal mucosa, the activation of pattern recognition receptors (PRRs) initiates regulation pathways, such as nuclear transcription factors-κB (NF-κB) and Mitogen-activated protein kinases (MAPK) pathways, to produce a variety of cytokines and chemokine (Sharma, Young, & Neu, 2010). Some PRRs ligands are receptors of probiotics or symbiotic bacteria, so that intestinal mucosal response to these bacteria does not cause inflammation. Meanwhile, most probiotics can prevent the degradation of the inhibitor of κB (IκB) proteins, which negatively regulate the NF-κB transcription factor or use peroxisome proliferator-activated receptor gamma (PPAR γ) to conduct NF-κB p65 subunit to limit NF-κB pathway (Ganguli & Walker, 2012). In addition, probiotics can promote regulatory T cells to secrete immune cytokines IL-10 and TGF and regulate Th1 and Th2 responses; thereby affecting the T mediated immune homeostasis (Kelly, Conway, & Aminov, 2005). Schultz et al (2003) have found in healthy subjects, probiotic *Lactobacillus rhamnosus* GG (LGG) can cause the synthesis of anti-inflammatory interleukin IL-10 and inhibit pro-inflammatory substances IFN-γ, IL-6, and TNF-α secreted by CD4 +T cells through intestinal microbial stimulation (Schultz et al., 2003). Moreover, like probiotics regulating immune cells to produce cytokines that promote or respond to inflammation, prebiotic IMD can enhance immunity by controlling the expression of pro-inflammatory cytokines and decrease inflammation in the mouse colonic tissue.
Majumder et al. have found IMD treatment significantly reduces the expression of TNF-α and IL-6 and furthermore may weaken the TNF-α-induced inflammatory responses by inhibiting or reducing the up-regulation of TLR-4 (Majumder et al., 2017). Pro-inflammatory molecules like LPS activate TLR4-mediated NF-κB signaling pathway. Therefore, prebiotic IMD may exhibit the anti-inflammatory mechanism by reducing the expression of TLR-4. Besides, prebiotics have been found to be a master regulator involved in activation of Kupffer cells to release various products, including cytokines, prostanoids, nitric oxide and reactive oxygen species, thus modulating immune activity in liver macrophages (Neyrinck, Alexiou, & Delzenne, 2004). The immune activity of the probiotics is achieved by interacting with their receptors to activate signal transduction molecules, such as NF-κB, that stimulates immune cells. Although the specific prebiotic IMD receptor has not yet been identified, β-glucan and mannose receptors have been identified on the immune cells (Song et al., 2014). In vitro study has shown that fructose can alter the phagocytosis of non-opsonin, suggesting fructose receptors are present in immune cells (Manzanares & Hardy, 2008). At present, the preventative mechanism of IMD on inflammation has not been confirmed and further studies are required to identify other possible IMD anti-inflammatory activities in LPS-induced inflammatory mice.

5.3 Short Chain Fatty Acids Analysis

In the current study, the intestinal microenvironment changed under the inflammatory condition, including the type and quantity of intestinal flora, could influence the level of SCFAs in the intestinal tract. The significantly higher SCFA concentrations in treatment groups compared with negative and positive control groups suggest that colonic fermentation differs between the groups and the treatment of probiotic *L. pentosus* S-PT84 and prebiotic IMD. It has been suggested that increased energy harvest from colonic fermentation is associated with a
higher Firmicutes: Bacteroidetes ratio and may impact the SCFAs production as has been seen in animal studies (Turnbaugh et al., 2006). Treatment group mice with a higher proportion of *B. bifidum* and *L. casei* had a stronger fermentation capacity and thus obtained a higher yield of SCFAs. At the same time, the production of SCFAs from bacterial fermentation of carbohydrates decreased the intestinal pH, which improved gut microbiota composition by facilitating the beneficial bacterial colonization and suppressing pathogenic bacteria invasion (Tuohy et al., 2005). The cooperation between bacteria and SCFAs ensures the stability of the intestinal flora composition and the intestinal environmental homeostasis.

Most importantly, as SCFAs are one of the important energy sources of the GI tract, the additional SCFAs may maintain the structure and function of gut (Peterson & Artis, 2014). Current studies have confirmed that SCFAs can maintain the mucosal morphology of the small intestine, alleviate the damage of intestinal epithelial cells, and protect the intestinal mucosal barrier (Comalada et al., 2006; Tlaskalova-Hogenova et al., 2011). Andoh, Tsujikawa, & Fujiyama, 2003) put the SCFAs on the transplanted small intestine of rats by parenteral nutrition and observed the morphological changes of the small intestinal mucosa after 10 days. It was shown by transmission electron microscopy that the villus height, crypt depth and mucosal thickness of the intestinal mucosa in SCFAs group were significantly higher than those in the control group (Andoh, Tsujikawa, & Fujiyama, 2003). Therefore, higher concentrations of SCFAs of treatment group mice might participate in enhancing colon morphology and intestinal physiological function. In addition, SCFAs are directly involved in modulating proliferation and differentiation of colon epithelial cells (Liong, 2008). An increased colonic mucosal cell proliferation index treated with SCFAs was identified in the colon of the rat model of postoperative chemotherapy compared with the control group, which indicated the addition of
SCFAs could promote the colonic mucosal cell proliferation in postoperative treatment rats (Stoidis et al., 2011). These results suggest the production of SCFAs can promote the proliferation of colonic mucosal cells, reduce the atrophy of intestinal mucosa, and maintain the morphology of intestinal mucosa.

As SCFAs may protect the epithelial cells of the small intestine and colon, which has a profound influence, as these cells are regulators of barrier function and immune homeostasis (Peterson & Artis, 2014). Butyric acid as the main SCFAs can reduce the glutamine demand of epithelial cells and alter gene expression of epithelial cells (such as IL-8 and monocyte chemotactic protein 1), which in turn changes signal transfer of epithelial cells to the mucosal immune system (Gibson et al., 2004). It has been confirmed that SCFAs affected by enteritis are mainly butyric acid and propionic acid and the intestinal inflammation can be significantly alleviated by the treatment of butyric acid after the experimental animal induced enteritis (Mishiro et al., 2013). In order to determine the immune activity of butyric acid, Canani et al (2011) applied sodium butyrate to colon cells in rat models of colitis to detect apoptosis of colon epithelial cells. They have found that compared with the normal group, the colon cell apoptosis rate of sodium butyrate group is significantly lower than normal group, which declares normal concentration of sodium butyrate in the intestinal cavity can inhibit the apoptosis of normal colonic epithelial cells in vitro (Canani et al., 2011). Additionally, SCFA receptors like G protein coupled receptors 41 and 43 bind to the immune cells of gut associated lymphoid tissue (GALT), which protects the host from invasion in the gut. The activation of GPR41 and GPR43 in intestinal epithelial cells by SCFAs also triggers mitogen-activated protein kinase pathway and cytokines and chemokine production, which are closely related to protective immunity and tissue inflammation in mice (Kim et al., 2013). These effects that SCFAs have on epithelial cells state
that SCFAs play an important role in maintaining intestinal homeostasis, enhancing intestinal mucosal self-repair and participating in intestinal immune regulation.

Moreover, SCFAs acts as an anti-inflammatory agent by influencing the release of cytokines by certain inflammatory cells (Cavaglieri et al., 2003). At present, many studies have found that SCFAs have some influence on the formation of inflammatory factors, making it a research hotspot (Vonolo et al., 2011; Sellin et al., 2014). As previously described, the epithelial cells of the small intestine and colon protected by the SCFAs are involved in producing pro-inflammatory cytokines. Butyrate acid also has an inhibitory effect on pro-inflammatory cytokine-induced NF-κB activation. To confirm the relationship between SCFAs and anti-inflammatory cytokine release, Tedelind et al (2007) have found acetic acid, propionic acid, butyric acid can reduce the TNF-α release without affecting the release of IL-6 protein (Tedesilind et al., 2007). All types of SCFAs have a particular therapeutic effect on inflammatory response, but there are differences in efficacy. Propionate and butyric acid have an equivalent effect of reducing the release of IL-6 protein from cultured organs, while acetic acid is less useful (Cavaglieri et al., 2003). By changing the type or the amount of cytokine or chemokines produced by intestinal cells, SCFAs may alter the pattern of inflammatory mediators produced in different tissues (Vinolo et al., 2011). In addition, SCFAs also help regulate pro-inflammatory cytokine abundance in the small intestine, while not adversely impacting plasma pro-inflammatory cytokine levels or causing small intestinal inflammation. Milo et al., (2002) have performed SCFAs as the supplement for total parenteral nutrition to identify their effect on pro-inflammatory cytokine levels in porcine. It is worth pointing out that increased small intestinal pro-inflammatory cytokine abundance induced by SCFAs is not associated with an elevated systemic concentration of pro-inflammatory cytokines (Milo, Reardon, & Tappenden, 2002).
6. Conclusion and Future Research

The present study has determined that LPS-induced chronic low-grade inflammation changes the composition and quantity of gut microbiota in mice. The restorative effects of probiotics and prebiotics on gut microbiota homeostasis are prominent as the proportion of B. *bifidum* and *L. casei* increase while *E. coli* and *C. difficile* had a smaller proportion. LPS administration to mice damaged gut tight junction, which was associated with increased gut permeability and gut microbiota translocation. Meanwhile, LPS permeated through a leaky gut into the bloodstream and tissues. As expected, the supplement of probiotic *L. pentosus* S-PT 84 and prebiotic IMD stimulated the growth of beneficial bacteria and thus enhanced competitiveness with pathogens, mucosal barrier function and inhibition of bacterial translocation, ultimately ameliorating gut dysbiosis and improving intestinal microflora ecology.

Future research should continue to investigate the effects of probiotic *L. pentosus* S-PT 84 and prebiotics IMD on gut microbiota and barrier function. It could include assessing the impact of probiotics and prebiotics on different species of microbiota in mice gut, culturing microbiota with probiotics and prebiotics to confirm their ability on the reestablishment of gut microbiota, and observing the efficacy of probiotics and prebiotics on inhibition of the LPS-activated TLR4 signaling pathways.

Future work should also focus on the anti-inflammatory activity of probiotic *L. pentosus* strain S-PT84 and prebiotic IMD in a mouse model of LPS-induced inflammation. Low-grade inflammation is associated with the elevated concentrations of pro-inflammatory molecules and such persistent situation may cause insulin resistance and other metabolic diseases (Heilbron & Campbell. 2008). Probiotics and prebiotics may be considered as a potential candidate for preventing inflammatory symptoms and its related metabolic disorders. There is some evidence
provided endorsing the pro-inflammatory action of SCFAs in some conditions, therefore it is also necessary to understand the role of SCFAs on the interaction between bacteria and host immune cells \textit{in vivo} (Vinolo et al., 2009; Smith et al., 2013).
7. References


intestine morphology, and resident *Lactobacillus* of male broilers. *Poult Sci*, 95(6), 1332-1340.


