Targeting gut microbiota: Lactobacillus alleviated type 2 diabetes via inhibiting LPS secretion and activating GPR43 pathway

Kai-Kai Li a, Pei-Jun Tian b, Shao-Dong Wang c, Peng Lei a, Ling Qu a, Jia-Peng Huang c, Yu-Juan Shan a,* Bao-long Li c,*

a Department of Food Science and Engineering, Harbin Institute of Technology, Harbin, China
b School of Food Science and Technology, Jiangnan University, Wuxi, China
c Center of Drugs Safety and Evaluation, Heilongjiang University of Chinese Medicine, Harbin, China

ABSTRACT

Lactobacillus G15 and Q14, two species of Lactobacillus used in the preparation of Chinese traditional fermented dairy foods, have the potential to prevent diabetes. Herein, we clarified their mechanisms by which gut microbiota relate to the control of diabetes. Results showed that G15 and Q14 improved the disorders in blood glucose and insulin. Moreover, G15 and Q14 promoted the enrichment of SCFA-producing bacteria, and upregulated the production of acetate and butyrate. Their antidiabetic effects were closely associated with SCFAs-downstream receptors and hormone secretion. Additionally, G15 and Q14 significantly shortened the number of G-negative bacteria, which subsequently decreased the levels of LPS and inflammatory factors. G15 and Q14 improved the disorganization of the intestinal mucous and the integrity of intestinal barrier. Our results indicate that Lactobacillus G15 and Q14 alleviate type 2 diabetes in a gut microbiota-dependent way via downregulating G/C0 bacteria-related LPS secretion, as well as upregulating SCFAs-producing bacteria-related G protein-coupled receptor 43 pathway.

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1. Introduction

Gut microbiota, a predominant component of intestinal barrier, is a part of a dynamic ecosystem in our body. Its composition can be altered at the phylum and class levels by many endogenous and exogenous factors including genetic features, diet, environmental microbial exposures and the physiological and pathological states of the host. Accumulated studies in recent years have shown that gut microbiota dysbiosis is closely linked with a wide spectrum of disease, including cancers, obesity, diabetes, and even mental illness (Human Microbiome Project & C, 2012; Qin et al., 2010).

Due to the crucial roles of gut microbiota in these diseases, treatments targeting gut microbiota has been paid much attention. On the basis of studies in both rodents and humans, dietary intervention appears to be a major short-term and long-term regulator of the gut microbiota, especially by prebiotics and probiotics. In recent years, prebiotics and probiotics have been increasingly used for exploring functional foods and dietary supplements (Tian et al., 2016). Prebiotics, defined as a nonviable food components that confer a health benefit by modulation of the composition of gut microbiota in the host (Pineiro et al., 2008). Accumulating preclinical and clinical evidence suggests that prebiotics have therapeutic effects on metabolic diseases. In one such study, fifteen obese volunteers were fed inulin-type fructans (ITF prebiotics), which led to subtle changes in their gut microbiota that could impact on several key metabolites implicated in obesity and/or diabetes (Dewulf et al., 2013). In addition, in diet-induced or genetic models of obesity in rodents, ITF were shown to decrease body weight gain and fat mass accumulation, improve glucose tolerance and insulin resistance (Cani, Neyrinck, Maton, & Delzenne, 2005; Cani et al., 2006; Cani et al., 2007; Cani et al., 2009). Probiotics are defined as ‘live microorganisms which when administered in adequate amounts, confer a health benefit on the host’. Studies show that increased probiotic consumption correlated with improved glucose tolerance and insulin secretion (Yadav, Jain, & Sinha, 2007; Yin, 2010).

Recently, literature has highlighted the relationship between the gut microbiota and T2D (Karlsson et al., 2013; Larsen et al., 2010; Qin et al., 2012; Sato et al., 2014). Although there are few clinical studies focused on probiotics and T2D, the antidiabetic effects of probiotics have been validated in many animal experiments. However, the prospects of probiotic application in T2D have...
not been extensively explored, and the molecular mechanisms involving the antidiabetic effects of probiotics are not fully elucidated but it may be related to restoration of gut permeability, modulation of inflammatory response, attenuation of inflammation, and modification of the gut microbiota (He, Shan, & Song, 2015).

Our previous studies in vivo have demonstrated that *Lactobacillus paracasei* subsp. *paracasei* G15 and *Lactobacillus* casei Q14, two kinds of probiotics extracted from Chinese traditional fermented foods, present beneficial effects on the management of diabetes-related parameters such as body weight and blood glucose (Tian et al., 2016). Also G15 and Q14 appear to normalize the gut dysbiosis in T2D rats. We postulated that gut microbiota may be a key target through which G15 and Q14 improved the T2D-related parameters. Therefore, the current study aims to elucidate the underlying mechanisms. Our findings indicate that two individually regulatory pathways linking to intestinal flora may be involved in the anti-diabetic actions of G15 and Q14.

2. Materials and methods

2.1. Ethics statement

All procedures involving animals and their care were conducted in conformity with guidelines of the Institutional Animal Care Use Committee, Heilongjiang province, China. The protocols were approved by Ethics Committee of Experimental Animals (Qualified number: SCXK-Hei-2012-016) at Heilongjiang University of Chinese Medicine.

2.2. Regents and materials

*Lactobacillus* G15 and Q14 were separated from Chinese traditional fermented diary food. G15 was isolated from Yak yoghurt in Gansu Province and Q14 from Yak yoghurt in Qinghai Province of China. The strains were identified by 16S rRNA gene sequences and stored at our lab. Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The animal chow was purchased from Keaoxieli Feed Co., Ltd (Beijing, China). Metformin was obtained from Sino-American Shanghai Squibb Pharmaceuticals Ltd (Shanghai, China). Glucagon-like peptide-1 (GLP-1) and Peptide YY (PYY) were measured using the Milliplex gut hormone panel. Lipopolysaccharide (LPS), total cholesterol, triacylglycerol kit was obtained from Jiaocheng Bioengineering Institute (Nanjing, Jiangsu, China). Stool DNA kit was purchased from Omega Bio-Tek (Norcross, GA, USA). TransStart Top Green qPCR SuperMix kit, TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix kit were purchased from TransGen Biotech (Beijing, China). The antibody was obtained from Proteintech Group, Inc (Wuhan, Hubei, China).

2.3. Preparation of Lactobacillus suspensions

As previously described (Tian et al., 2016), *Lactobacillus* G15 and Q14 strains were cultured in MRS broth with 0.05% (w/w) L-cysteine and incubated at 37 °C under anaerobic conditions. Oral administration samples were prepared by suspending lyophilized bacteria powder with sterile saline. Colony counting was performed to obtain the numbers of surviving bacteria and to adjust the final cell concentration to 4 × 10⁸ cfu ml⁻¹.

2.4. Animals and experimental design

Experimental design was as previously described (Tian et al., 2016). Male Wistar rats, weighing 160–200 g, specific pathogen free, were obtained from Center of Drugs Safety and Evaluation of Drugs, Heilongjiang University of Chinese Medicine. Animals were housed in a controlled-environment (temperature 22 ± 2 °C and humidity 55 ± 5% with 12/12 h light and dark cycle) with free access to food and water. The high-fat diet (HFD) contains 45% fat, 35% protein and 20% carbohydrate (a percentage of total kcal, according to D12451; Research Diets Inc.) while the normal chow consisted of 15.8% fat, 20.3% protein and 63.9% carbohydrate (according to the AIN-93G diet). The formula of diet is shown in Table S1. Diabetic group models were established using the HFD for 6 weeks followed by a low-dose STZ injection as in previous studies (Manaer, Yu, Zhang, Xiao, & Nabi, 2015; Sririnivasan & Ramarao, 2007). Rats in the negative control group were fed on normal chow. At the 7th week, rats in the diabetic group were intraperitoneally injected with STZ (35 mg kg⁻¹, dissolved in 0.05 M sodium citrate buffer, pH 4.5) and the normal control group with vehicle citrate buffer (0.5 mL kg⁻¹). Fasting blood glucose was measured on the 3rd and the 7th days after the injection. Rats with glucose levels >11.1 mmol/L were defined as T2D. The T2D rats were then randomly divided into 4 groups and fed on the normal chow until 13th week. Five groups (n = 8 each) were identified as follows: Normal control group (N); Diabetes control group (D); Metformin treated group (M); *Lactobacillus* G15 treated group (G); *Lactobacillus* Q14 treated group (Q). Metformin and *Lactobacillus* doses were administered by oral gavage. The experimental scheme is shown in Fig. 1.

2.5. Oral glucose tolerance test (OGTT)

Rats were fasted overnight and administered with a solution of 40% glucose (2 g/kg) by oral gavage. Tail blood samples were collected at 0, 30, 60, 90 and 120 min after glucose load, and blood glucose concentrations were measured by a glucometer (Roche Diagnostics, Germany). The area under curve of glucose (AUC-glucose) values for 0–120 min post glucose load was calculated using ORIGIN 9.0.

2.6. Serological indicators measurement

Blood samples were collected from abdominal aorta, centrifuged at 3000g for 15 min, plasma and serum were carefully removed from the edetic-acid-containing and plain tubes respectively and stored at –80 °C. LPS, Insulin, Glucagon, Total cholesterol, Triacylglycerol, GLP-1 and PYY were quantified by ELISA kits according to the manufacturer’s protocols.

2.7. Analysis of intestinal flora by real-time quantitative PCR

Faecal microbiota stool samples were randomly collected from rats that were fasted for 20 h. Stools were consecutively and aseptically collected for three days at the 6th week (for baseline) and 13th week (the end of experiment), then mixed evenly into three samples each group and stored at –80 °C for the extraction of DNA. The DNA was extracted by a stool DNA kit according to manufacturer instructions. The specific primers of total bacteria, Gram-positive (G⁺) bacteria, Gram-negative (G⁻) bacteria, *Bifidobacterium*, *Lactobacillus*, *Clostridium leptum*, *Bacteroides Prevotella*, *Bacteroidetes*, *Enterobacterium* were designed according to the 16srRNA V3 sequence of BLAST gene library (www.ncbi.nlm.nih.gov/BLAST). The sequences of primers are shown in Table S2. The primers were synthesized by Dalian Bao Biotechnology Co., Ltd. DNA was then subjected to quantitative PCR using Quantifast SYBR Green PCR kit. Results are expressed as bacteria number per g of stool.
2.8. SCFAs concentrations quantified by gas chromatography (GC)

The short-chain fatty acids (SCFAs) including acetate, propionate, butyrate, isobutyrate, valerate and isovalerate in the feces were analyzed by GC. Fecal samples (200 mg) were collected directly into sterile tubes from live rat and were homogenized in 1 mL of sterile water and then oscillated for 10 min. The suspension was centrifuged at 18,800 × g for 10 min and the supernatant was again centrifuged at 18,800 × g for 15 min. The supernatant was subjected to 0.45 μm membrane filtration. Filtrate (1 mL) and formic acid (0.1 mL) were taken into a gas-phase flask for the analysis of the SCFAs.

2.9. RNA extraction and real-time quantitative PCR

After isolation of total RNA using TRIZOL reagent kit (TransGen Biotech, China) and quantification by measuring optical density at 260 nm and 280 nm, cDNA was synthesized by Transcript One-Step g DNA Removal and cDNA Synthesis SuperMix kit (TransGen Biotech, China) according to the manufacturer’s protocol. The first strand obtained was quantified by real-time quantitative PCR using a Passive Reference Dye I (50 μl/C2 assay) on the ABI Prism 7300 System (Applied Biosystems, CA). The sequences of primers are shown in Table S3. The PCR cycle conditions for human gene consisted of 94°C for 31 s, then 94°C for 5 s and 60°C for 30 s (40 cycles).

2.10. Histopathologic examination (HE) and immunohistochemistry

The anesthesia position of colon was confirmed according to the previous description (Wang et al. Chief editor, 2009). Briefly, colonic tissue (around 1 cm-length) was selected in the descending colon (about 5 cm from the anus), and cleaned with saline. Then colons were fixed in 10% formalin saline for 48 h, dehydrated in a series of graded alcohols (100, 95 and 80%) and embedded in paraffin, serially cut at 4 μm thickness with a rotary microtome (Leica, RM2235, Germany). Slides without HE staining were hybridized with the appropriate secondary antibody (1:500 dilution) at room temperature for 1 h. Images were captured using light microscopy (ZEISS, Axio vert.A1, Germany). The integrated optical density (IOD) analysis was quantified by Image-Pro plus 6.0.

2.11. Statistical analysis

All data are represented as mean ± SEM. SPSS 19.0 software was used for statistical analysis. Statistical significance of difference was determined using one way ANOVA followed by multiple comparisons with a Tukey’s test. The difference was considered statistically significant at \( P < 0.05 \).

3. Results and discussion

3.1. Effects of G15 and Q14 on body weight, glucose tolerance and insulin

The rodent T2D model was established by a combination of short term HFD feeding and low dose of STZ (35 mg·kg\(^{-1}\)·ip) injection which mimicked the natural history and intrinsic features of T2D in human beings. Body weight consequently increased during the experimental period (Fig. S1). STZ injection (at the beginning of week 7) caused a significant weight loss in all HFD groups from week 8 to week 10, which was similar to recent research studies showing that STZ induction of type 2 diabetes led to decreased body weight (Liu, Sun, Rao, Su, & Yang, 2013). G15 and Q14 treatment reduced, to some extent, the decrease in body weight induced by STZ injection. This effect was also observed in the M group. (Fig. S1A). A previous study found that HFD-induced body weight increase was prevented by probiotics (L. plantarum K68) (Huang, Korivi, Tsai, Yang, & Tsai, 2013), which was consistent with our results.

Glucose intolerance is a well-established diagnostic criterion for insulin resistance in T2D. At the end of week 7, glucose tolerances were impaired in the HFD-STZ groups (Fig. 2A) and the AUC-glucose values were more than 3 times higher (Fig. 2a) than in the N group. After the 6-week treatment, the glucose intolerance was effectively restored in all treatment groups (Fig. 2B & b), although still higher than the normal level. These results indicated that G15 and Q14 could efficiently improve glucose tolerance and prevent the development of hyperglycemia in T2D. Oral administration of Lactobacillus rhamnosus CCFM0528 has resulted in improved glucose tolerance in HFD, STZ-induced T2D mice (Chen et al., 2014), which was consistent with our results. Additionally, G15 and Q14 suppressed HFD-induced total cholesterol (Fig. S1B) and triacylglycerol level (Fig. S1C).

Insulin and glucagon are sensitive parameters for diagnosing diabetes and an indicator of glycemic control (Chowdhury et al.,...
2017), Insulin and glucagon concentration reached the highest levels in the D group (Fig. 2C and D) and notably, G15 and Q14 largely decreased the insulin and glucagon concentrations (Fig. 2C & D). Our results indicate that G15 and Q14 improved hyperinsulinemia. In large population samples, insulin resistance and associated metabolic abnormalities have been estimated to be present in 60–80% of patients with T2D (Natali et al., 2006).

3.2. G15 and Q14 promoted the enrichment SCFA-producing bacteria and SCFAs

It has been suggested that short chain fatty acids (SCFAs) produced by the gut microbiota play a pivotal role in regulating host metabolism (Fernández et al., 2016). Recently, the detailed interactions between the beneficial gut bacteria (e.g., SCFA-producing bacteria) and opportunistic pathogens (e.g., endotoxin-producing bacteria) have emerged as crucial factors for metabolic homeostasis in T2D (Qin et al., 2012). In the present study, acetate-producing bacteria, such as Lactobacillus and Bifidobacterium, were markedly decreased in the D group but G15 and Q14 treatments largely improved them (Fig. 3A & B). Importantly, SCFA-producing bacteria such as Clostridium leptum (mainly produce butyrate), Bacteroides Prevotella (mainly producing acetate and butyrate) were markedly increased by G15 and Q14 (Fig. 3C & D). The similar results which addressed the beneficial effects of probiotics on the composition of gut microbiota were also observed in other literatures (Aoki et al., 2017; Ishizuka et al., 2012; Louis & Flint, 2007). Moreover, the major butyrate producers, such as Roseburia and Faecalibacterium prausnitzii, are less abundant in T2D; but they are well known for butyrate synthesis and are associated with improved insulin sensitivity (Louis & Flint, 2007; Louis & Flint, 2009).

Numerous physiological and clinical studies have demonstrated that the administration of SCFAs resulted in a wide range of health benefits including improvements in body composition, glucose homeostasis, and blood lipid profiles (Byrne, Chambers, Morrison, & Frost, 2015). Combined with the above findings, we postulated...
that the improvements of blood glucose and glucose intolerance may be related to the acetate and butyrate-producing bacteria in the gut. Consequently, the production of a variety of SCFAs were determined in feces. Lower acetate and butyrate concentrations were observed in the D group. In contrast, G15 and Q14 increased acetate and butyrate concentrations (Fig. 3a & c), but these were still lower than the normal level. There were no statistical differences in propionate, isobutyrate, valerate and isovalerate concentrations (Fig. 3b & d–f). These results provide evidence that intestinal microbial alteration could lead to changes in SCFAs metabolism. Indeed, it has been recently demonstrated that the enrichment of *Lactobacillus* and *Bifidobacterium* in BlaG-treated mice plays a major role in accelerating the production of SCFAs in the gut (Aoki et al., 2017). In addition, supplementation with propionate or butyrate separately improved body weight gain and glucose tolerance in rodents, with no associated increase of insulin secretion (De Vadder et al., 2014).

### 3.3. G15 and Q14 regulated blood glucose via upregulation of GRP43 and the secretion of GLP-1/PYY

SCFAs conferred some of their biological effects via the G protein-coupled receptors (GPRs), known as GPR43 and GPR41. Much attention has been paid to the beneficial roles of GPR43 in energy and glucose homeostasis (Kimura et al., 2013; Tolhurst et al., 2012). To establish and explore the link between SCFAs, G protein-coupled receptors and GLP-1 secretion, expression of GPR43 and GPR41 in the colonic tissue was investigated by quantitative RT-PCR. As shown in Fig. 4, the expression of GPR43 was decreased by 50% in the D group. Unexpectedly, G15 and Q14 significantly increased the expression of GPR43 (Fig. 4B) by about 1.5-fold. However, there was no significant change to the expression of GPR41 (Fig. 4A). An earlier study indicated SCFA activated GPR43 and enhanced GLP-1 secretion from primary colonic (Tolhurst et al., 2012), which is similar to our results. However, other studies suggested that SCFAs triggered signaling cascades by activation of GPR43 and GPR41 (Brown et al., 2003; Le Poul et al., 2003). GPR43 reportedly coupled to Gq- or Gi/0-signaling pathways and GPR41 exclusively to Gi-signaling pathway, a dominant role of GPR43 in SCFA-triggered L-cell activation was demonstrated by the acute Ca2+-elevations seen in primary L cells, which reportedly does not have activity against GPR41 (Lee et al., 2008). Alternatively, this regulatory difference may be due to the different types of SCFAs.

The combination of SCFAs and GPRs will stimulate the secretions of gut hormones downstream. Therefore, two antidiabetic gut hormone and peptides, GLP-1 and PYY, were determined by ELISA assay (Fig. 4C & D). Lower GLP-1 and PYY secretions were observed in the D group. G15 and Q14 largely augmented the concentration of GLP-1 and PYY, with similar regulation by metformin treatment. Consistent with the above, antidiabetic gut hormone and peptides, in particular, are associated with improved blood glucose control and reduced incidence of hypoglycemia but also with significant weight reduction (Madsbad, 2009). Previous reports demonstrated that GPR43 expressed in the intestine improves glucose tolerance by promoting the secretion of GLP-1 from L cells (Lee et al., 2008). Alternatively, this regulatory difference may be due to the different types of SCFAs.

The therapeutic effects of prebiotics on metabolic diseases, such as T2D, have been confirmed in a rodent model (Yadav et al., 2007). However, the regulation of probiotics in host metabolism through the short-chain fatty acids, G-protein coupled receptors and downstream hormones was scant. The present study demonstrated G15 and Q14 improved the T2D-related parameters in rats through specific microbiota-induced SCFAs signaling pathway. GPR43

![Fig. 3.](image)

**Fig. 3.** G15 and Q14 rectified the imbalance of intestinal flora and augmented the production of acetate and butyrate. Faecal microbiota stool samples were randomly collected from rats in the aseptic way. The DNA was extracted by a stool DNA kit and subjected to the quantitative PCR. The content of bacteria (A) *Lactobacillus*, (B) *Bifidobacterium*, (C) *Clostridium leptum*, (D) *Bacteroides Prevotella*. Concentration of SCFAs in the feces of male Wistar rat was performed by GC. (a) acetate, (b) propionate, (c) butyrate, (d) isobutyrate, (e) valerate, (f) isovalerate. Data are represented as mean ± SEM. significance was determined by one-way ANOVA corrected for multiple comparisons with Tukey’s test. Compared with N groups, ***P < 0.001****P < 0.0001, Compared with D group, *P < 0.05**P < 0.01***P < 0.001****P < 0.0001, ns-no significance.
expression in intestinal L-cells is stimulated by SCFAs, and also
induced the secretion of GLP-1, a gut-derived peptide known to
modulate satiety and glucose homeostasis (Kjems, Holst,
Volund, & Madsbad, 2003; Tolhurst et al., 2012; Zander,
Madsbad, Madsen, & Holst, 2002).

3.4. G15 and Q14 lowered the abundance of G−/C0 bacteria and the
concentration of LPS

Endotoxin has been recognized as a novel factor triggering the
onset of type 2 diabetes induced by a high-fat diet. In T2D, endoge-
 nous LPS were continuously produced in the gut by the aggravation
of G−/C0 bacteria (Cani et al., 2009). The present study showed that
the dominant members of G− bacteria, not G+ bacteria, were signif-
icantly different between all experimental groups (Fig. 5A–C). G15
and Q14 also lowered the concentrations of two typical G− bacte-
ria, Bacteroides and Enterobacterium. Taken together, G15 and Q14
largely rectified the imbalance of the gut microbiota in T2D rats
(Fig. 5D & E). Higher plasma LPS levels in the D group reected
the elevated intestinal permeability. G15, Q14 and metformin
treatments significantly restored the integrity of the intestinal epithelial barrier (Fig. 7A–F). These results were ver-
ified by RT-PCR. G15 and Q14 treatment significantly reversed the
decline of ZO-1 and Occludin mRNA (Fig. 7G–H). However, there
was no significant differences in the expression of Claudin
(Fig. 7I). G15 and Q14 treatment also significantly inhibited the
decline of Muc2 (Fig. 7J). Previous studies also showed probiotics
promoted the secretion of gut hormone, which regulated the
expression of mucin to improve the intestinal mucosal barrier
function (Strowski & Wiedenmann, 2009). Data showed that a
high-fat diet dramatically increased intestinal permeability by a
mechanism associated with a reduced expression of epithelial tight
junction proteins such as ZO-1 and Occludin, although only a ten-
dency was observed for occludin (Cani et al., 2008). Another study
showed that Lactobacillus M1 strengthened the epithelial layer and
reduced intestinal permeability (Chen & Chen, 2013; Chen, Lee,
Hong, Hsieh, & Chen, 2013). Akkermansia spp. has been reported

Fig. 4. Effect of G15 and Q14 on G-protein-coupled receptors and gut hormone. G-protein-coupled receptors (A) GPR41 and (B) GPR43 in the colon were detected by RT-PCR.
Serum GLP-1 and PYY were detected by ELISA. (C) The concentration of GLP-1. (D) The concentration of PYY. Data are represented as mean ± SEM, significance was determined
by one-way ANOVA corrected for multiple comparisons with Tukey's test. Compared with N groups, *P < 0.05 ****P < 0.0001, Compared with D group, P < 0.05 ”P < 0.01 
****P < 0.0001 compared with the D group, ns-no significance.

3.5. G15 and Q14 improved the intestinal mucosa integrity and
expressions of tight conjunction proteins

Gut barrier damage may lead to the increased permeation of
substances such as G− bacteria-derived LPS into the mucosal layer
(Ahrne & Hagslatt, 2011; Turner, 2009). Probiotics promoted a
healthier intestinal microenvironment by improving intestinal bar-
rier structure (Cani et al., 2009). The histological features of colon
in each group are illustrated in Fig. 6A–E. In the N group, the
epithelial and mucosal structures were intact and no inflammatory
infiltration was observed (Fig. 6A). However, thinner and discon-
tinuous overlaying inner mucus layers were observed in the dia-
abetic group (Fig. 6B). Remarkably, the epithelial and mucosal
structure was normalized in the G, Q and M groups (Fig. 6C–E).
The pathological improvements were characterized by more inte-
gral mucosa and more goblet cells.

To further confirm whether G15 and Q14 regulate intestinal
barrier function, we analyzed the expression of tight junction pro-
teins zonula occludens-1 (ZO-1) in the colon by immunohisto-
chemistry. The mean integral optical density (IOD) value of ZO-1
was significantly decreased in the D group (Fig. 7F). G15, Q14
and metformin treatments significantly restored the integrity of
the intestinal epithelial barrier (Fig. 7A–F). These results were ver-
ified by RT-PCR. G15 and Q14 treatment significantly reversed the
decline of ZO-1 and Occludin mRNA (Fig. 7G–H). However, there
was no significant differences in the expression of Claudin
(Fig. 7I). G15 and Q14 treatment also significantly inhibited the
decline of Muc2 (Fig. 7J). Previous studies also showed probiotics
promoted the secretion of gut hormone, which regulated the
expression of mucin to improve the intestinal mucosal barrier
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high-fat diet dramatically increased intestinal permeability by a
mechanism associated with a reduced expression of epithelial tight
junction proteins such as ZO-1 and Occludin, although only a ten-
dency was observed for occludin (Cani et al., 2008). Another study
showed that Lactobacillus M1 strengthened the epithelial layer and
reduced intestinal permeability (Chen & Chen, 2013; Chen, Lee,
Hong, Hsieh, & Chen, 2013). Akkermansia spp. has been reported
to increase the production of caecal mucin induced by dietary carbohydrates (Morita, Tanabe, Sugiyama, Kasaoka, & Kiriyama, 2004).

3.6. G15 and Q14 diminished the secretion of inflammatory cytokines

Studies have shown that LPS binding to the complex of CD14 and TLR4 at the surface of innate immune cells triggers the secretion of pro-inflammatory cytokines, such as IL-1β, IL-8, IL-6 (Jialal, Huet, Kaur, Chien, & Devaraj, 2012; Liang, Hussey, Sanchez-Avila, Tantiwong, & Musi, 2013; Saito, Hayashida, & Furugen, 2007; Wellen & Hotamisligil, 2005), which leads to pancreatic β-cells destruction. Our previous research has shown that G15 and Q14 treatment improved the histological abnormalities with enriched normal β-cells and acinar cells (Fig. S2A-E) (Tian et al., 2016). The present study results showed that the concentrations of IL-
1β, IL-8 and IL-6 were raised in the D group (Fig. 8A–C). G15 and Q14 treatments significantly lowered IL-1β, IL-8 and IL-6 levels. Evidence suggests that these inflammatory responses initiated by LPS in the host are mediated through toll-like receptor signaling pathways (Liang et al., 2013). Several serine/threonine kinases, which contribute to inhibition of insulin signaling (JNK, IKK), were then activated by inflammatory factors and thereby inhibit insulin action followed by insulin resistance (Wellen & Hotamisligil, 2005). This study revealed that G15 and Q14 administration markedly suppressed the increased concentrations of IL-1β and IL-8 in serum, but no significant differences were previously observed in the levels of IL-6 (Tian et al., 2016). Thus, the data from this study suggests that administration of G15 and Q14 could induce colonic IL-1β, IL-8 and IL-6 production.

The above results show that GPR43 expression stimulated by SCFAs has improved glucose tolerance by promoting the secretion of glucagon-like peptide-1 from L cells. Additionally, G15 and Q14 improved insulin resistance which contributes to the development
of inflammation. Kimura et al. reported that GPR43 signaling in adipocytes inhibits insulin signaling. Moreover, it has been reported that GLP-1 and GPR43 signaling potentiated insulin secretion by beta cells in the pancreas (Kimura et al., 2013; Kjems et al., 2003) and a further report showed that insulin resistance could be improved through toll-like receptor signaling pathways (Wellen & Hotamisligil, 2005). Most recently, microbiota metabolite SCFA acetate promoted intestinal IgA response, which has a crucial role in maintenance of intestinal homeostasis and in protecting the intestines from inflammation through stimulating metabolite-sensing GPR43 (Wu et al., 2017). Therefore, GPR43 plays the indispensable roles both in insulin signal and intestinal homeostasis.

4. Conclusions

This study primarily studied the antidiabetic effect and mechanism of *Lactobacillus paracasei* subsp. *paracasei* G15 and *Lactobacillus casei* Q14. G15 and Q14 improved the T2D-related parameters in rats through the two major aspects, gut microbiota-related intestinal mucosal barrier and specific microbiota-related short chain fatty acids. The probiotic treatment repaired the glucose intolerance and reduced the serum lipid levels. G15 and Q14 also reduced intestinal mucosal permeability and improved epithelial barrier function through modification of tight conjunction proteins, eventually alleviating T2D associated inflammation and β-cell dysfunction. From the perspective of gut microbiota, G15 and Q14 promoted the enrichment of SCFA-producing bacteria and upregulated the production of short chain fatty acids. The antidiabetic effects were mediated by the pathway of SCFA-downstream receptors (GPR43) expression and hormone secretion (GLP-1, PYY). This study provides a detailed antidiabetic mechanism between gut microbiota and T2D. The findings further confirmed the potential of probiotics, derived from traditional foods, to improve the status of diabetes sufferers.

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Conflict of interest

The authors declare no competing financial interest.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2017.09.049.

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