

# Effect of heat-killed *Streptococcus thermophilus* on type 2 diabetes rats

Xiangyang Gao<sup>1,\*</sup>, Fei Wang<sup>1,\*</sup>, Peng Zhao<sup>1,2</sup>, Rong Zhang<sup>1</sup> and Qiang Zeng<sup>1</sup>

<sup>1</sup> Health Management Institute, The Second Medical Center of Chinese PLA General Hospital, Beijing, China

<sup>2</sup> Health Management Center, Hangzhou Special Service Convalescent Center of Air Force, PLA, Hangzhou, China

\* These authors contributed equally to this work.

## ABSTRACT

**Background and Aims:** The link between gut microbiota and type 2 diabetes (T2D) has been addressed by numerous studies. *Streptococcus thermophilus* from fermented milk products, has been used as a probiotic in previous research. However, whether heat-killed *S. thermophilus* can improve the glycemic parameters of diabetic rats remains unanswered. In this study, we evaluated the effect of heat-killed *S. thermophilus* on T2D model rats and the potential mechanisms of the effect.

**Methods:** Zucker diabetic fatty (ZDF) rats were used to generate a diabetic rat model induced by feeding a high-fat diet. Heat-killed *S. thermophilus* were orally administered to normal and diabetic rats for 12 weeks. Intestinal microbiota analysis, histology analysis, oral glucose tolerance test and measurement of inflammatory factors were performed.

**Results:** We found that heat-killed *S. thermophilus* treatment reduced fasting blood glucose levels and alleviated glucose intolerance and total cholesterol in diabetic ZDF rats. Additionally, heat-killed *S. thermophilus* increased the interleukin 10 while reducing the levels of lipopolysaccharide, interleukin 6, and tumor necrosis factor- $\alpha$  in diabetic ZDF rats. The heat-killed *S. thermophilus* treatment can normalize the structure of the intestinal and colon mucosal layer of diabetic rats. The characteristics of the gut microbiota in heat-killed *S. thermophilus*-treated and control rats were similar. At the genus level, the abundances of beneficial bacteria, including Ruminococcaceae, Veillonella, Coprococcus, and Bamesiella, were all significantly elevated by heat-killed *S. thermophilus* treatment in ZDF diabetic rats.

**Conclusion:** Our study supports the hypothesis that treatment with heat-killed *S. thermophilus* could effectively improve glycemic parameters in T2D model rats. In addition, the potential mechanisms underlying the protection maybe include changing the composition of gut microbiota, reinforcing the intestinal epithelial barrier and the immunity of the intestinal mucosa, decreasing the level of inflammation, and then reducing the insulin resistance.

Submitted 4 February 2019

Accepted 10 May 2019

Published 13 June 2019

Corresponding author

Qiang Zeng,  
zengqianghospital@126.com

Academic editor

Daniela Foti

Additional Information and  
Declarations can be found on  
page 18

DOI 10.7717/peerj.7117

© Copyright

2019 Gao et al.

Distributed under

Creative Commons CC-BY 4.0

OPEN ACCESS

**Subjects** Microbiology, Diabetes and Endocrinology, Metabolic Sciences

**Keywords** Diabetes, Gut microbiota, Heat-killed *Streptococcus thermophilus*, ZDF rats

## INTRODUCTION

Diabetes is a chronic metabolic disease and an important cause of mortality and morbidity worldwide, the prevalence of which is dramatically increasing. The number of adults with diabetes, mostly Type 2 diabetes (T2D), has increased to 422 million around the world (*NCD Risk Factor Collaboration (NCD-RisC), 2016*). Diabetes and its complications account for more than two million deaths every year (*Li et al., 2016*).

Recently, immense evidence has been obtained linking T2D and gut microbiota. The significant correlations with specific gut microbes, bacterial genes, and metabolic pathways in T2D patients were showed by a human metagenome-wide association study (*Larsen et al., 2010*). The data from animal and human models also suggest that T2D is associated with a moderate degree (*Qin et al., 2012*) to profound gut microbial dysbiosis (*Tilg & Moschen, 2014*). Increasing evidence indicates that gut microbiota are strongly associated with diabetes development (*Haro et al., 2015; Mejía-León & Barca, 2015*). Other studies even show that gut microbiota markedly contribute to the incidence of T2D (*Baathman et al., 2016; Tai, Wong & Wen, 2015*). The dysbiosis of gut microbiota may damage the intestinal epithelial barrier, and increase the intestinal permeability, and thus promotes metabolic endotoxemia, and systemic inflammation (*Prattichizzo et al., 2018; Winer et al., 2016*), leading to the development of insulin resistance (*Jorge et al., 2012; Boulange et al., 2016*), thereby increasing the risk of developing T2D (*Pedersen et al., 2016; Hartstra, Nieuwdorp & Herrema, 2016*). These studies suggest that the gut microbiota are potential targets for the treatment of T2D.

Probiotics have been proven to be effective in T2D. Administration of probiotics in a rat model effectively inhibited gluconeogenesis in T2D (*Amandine et al., 2013*). Treatments with probiotics have been demonstrated to be efficacious against tissue inflammation, and insulin resistance by modulating the gut microbial structure (*Moya-Pérez, Neef & Sanz, 2015; Shin et al., 2014*). However, the efficacy in T2D subjects varies, depending on the types and strains of probiotics.

Probiotics, as defined by the World Health Organization, are live microorganisms, that confer a health benefit to the host, when administered in adequate amounts (*FAO/WHO, 2001*). However, in many cases, probiotic preparations comprised of dead cells and their metabolites can also exert a biological response similar to that seen with live cells (*Dotan & Rachmilewitz, 2005; Sashihara, Sueki & Ikegami, 2006; Zhang et al., 2005*). For example, both live and heat-killed *Lactobacillus GG* had a similar anti-inflammatory effect (*Ehud et al., 2004*).

*Streptococcus thermophilus* is classified as a lactic acid bacterium, and it is found in fermented milk products, and generally used in the dairy industry (*Kilic et al., 1996*). *S. thermophilus* scavenges reactive oxygen radicals (*Lin & Yen, 1999; Bruno-Barcena et al., 2004*), thus demonstrating its antioxidant properties. *S. thermophilus* also shows immunomodulatory effects by stimulating the gut immune system (*Donkor et al., 2012; Delorme, 2008*). And *S. thermophilus* has been used as a probiotic to help prevent developing insulin resistance in previous research (*Asemi et al., 2013a*). However, to our knowledge, the question as to whether heat-killed *S. thermophilus* can improve glycemic

parameters remains unanswered. In addition, the potential mechanisms underlying the possible protection are still poorly understood. Therefore, the purpose of this research was to identify the beneficial effects of heat-killed *S. thermophilus* on diabetic rats and the potential mechanisms.

## MATERIALS AND METHODS

### T2D animal model

The Zucker diabetic fatty (ZDF) rats were used as a T2D model. ZDF rats have been an important model for studying the mechanism of treatment on T2D (Finegood *et al.*, 2001; Leonard *et al.*, 2005). Seven-week-old male ZDF rats were purchased from Charles River (Beijing, China). After 1 week of acclimation, diabetes was then induced by feeding a high-fat diet of Purina5008 (17% kcal fat and 26.5% kcal protein; IPS Supplies, London, UK) for 1 month. Then, 12-week-old male ZDF rats were obtained, and fasting blood glucose (FBG) >11.1 mmol/l was determined to be the standard concentration for the T2D model.

### Control rats

Seven-week-old male Sprague-Dawley (SD) rats also were obtained from Charles River (Beijing, China). After acclimating for 1 week, they were used as control rats.

Both the ZDF and SD rats were maintained at  $22 \pm 2$  °C with lights in an air-conditioned room with a 12-h light/dark cycle, and were given free access to food and water. A standardized diet (kcal%: 10% fat, 20% protein, and 70% carbohydrate) was administered. All of the experimental protocols were approved by the Animal Care Committee of the General PLA Hospital Animal Ethics Committee (Project CPLAGHAE-20171228-01).

### Study design

The diabetic ZDF rats were randomly divided into two groups: a heat-killed *S. thermophilus*-treated diabetic group (DM+ST, KAWAI; Kawai Lactic Acid Bacteria Research Institute Co., Ltd., Tokyo, Japan, orally administered 0.21 g Kawai powder/kg body weight/day,  $n = 5$ ) and an untreated diabetic group (DM, orally administered the same volume of normal saline,  $n = 5$ ). Kawai powder contains 28.75% heat-killed *S. thermophilus* and 20.60% resistant dextrin, 20.00% isomaltooligosaccharide, 17.00% microcrystalline cellulose, 10.00% xylo-oligosaccharides, 2.55% *Saccharomyces cerevisiae*, and 1.10% lemon juice powder.

Control rats were randomly divided into an untreated control group (CON, administered normal saline,  $n = 5$ ) and a heat-killed *S. thermophilus*-treated control group (CON+ST, orally administered 0.21 g Kawai powder/kg body weight/day,  $n = 5$ ). After treatment for 12 weeks, fresh stool samples were obtained by stimulating the anus, and they were frozen and stored at  $-80$  °C for subsequent analysis. After food deprivation for 12 h, the rats were anesthetized, blood samples were collected from the aorta abdominalis, and then the rats were sacrificed.

### Tissue collection and histology analysis

After rats were killed, the tissues of the ileum and colon were immediately excised, and then were cleaned with ice-cold phosphate-buffered saline solution. The tissues were fixed

in 4% formalin solution, then embedded in paraffin before being cut into four- $\mu\text{m}$  slices, followed by hematoxylin-eosin staining for measurement of villi length and crypt depth (10 villi and 10 crypts per section) under a light microscope (SZX16; Olympus, Tokyo, Japan).

### Western blot analysis

The ileum and colon tissues were homogenized in RIPA lysis buffer containing protease inhibitor cocktail (Roche, Indianapolis, IN, USA). Protein homogenates were separated on SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. After blocking for 1 h with 5% bovine serum albumin in Tris-buffered saline with 0.1% Tween (TBST: 50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.4), the membranes were incubated overnight with specific primary antibodies against Occludin (Abcam, Cambridge, UK), ZO-1 (Zonula occludens) (Santa Cruz Biotechnology, Dallas, TX, USA), and  $\beta$ -actin (Zsbio, Beijing, China) at 4 °C. Then, the membranes were incubated for 1 h with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (anti-rabbit or anti-mouse IgG-HRP) (Jackson ImmunoResearch Inc., West Grove, PA, USA), and the bands were detected by using enhanced chemiluminescence. The blots were scanned by a Bio-Rad ChemiDoc XRS and the intensity of each protein was quantified by Gel Image system V4.00 software (Tanon, Shanghai, China).

### Oral glucose tolerance test

At the end of the trial, an oral glucose tolerance test (OGTT) was performed after fasting for 12 h. Glucose (two g/kg body weight) was orally administered to the rats. The blood glucose levels which were obtained from the tail were recorded with a OneTouch UltraEasy glucometer (Johnson & Johnson, New Brunswick, NJ, USA) before and 15, 30, 60, 90, and 120 min after the glucose load. The area under the curve (AUC) was calculated by using the linear trapezoid method ([Zhang et al., 2016](#)).

### Measurement of inflammatory factors, serum insulin, lipid profile, HOMA-IR, and HbA1c

After food deprivation for 12 h, rat serum was obtained to analyze inflammatory factors (interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor (TNF)- $\alpha$ , and lipopolysaccharide (LPS) (ELISA, Elabscience, Wuhan, China), insulin (ELISA, Millipore, Billerica, MA, USA), total cholesterol (TC), triglyceride (TG, oxidase method; InTec Products, Fujian, China), high-density lipoprotein cholesterol concentrations (HDL-C), and low-density lipoprotein cholesterol concentrations (LDL-C, direct method, InTec Products, Fujian, China), according to the manufacturer's instruction. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by using the following formula:  $\text{FBG (mmol/l)} \times \text{fasting serum insulin } (\mu\text{IU/ml})/22.5$ . Rat plasma was also analyzed for HbA1c (Immunoturbidimetry; InTec Products, Fujian, China).

### Intestinal microbiota analysis

DNA extractions from total fecal bacteria were obtained using a QIAamp Stool DNA Extraction Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The microbial 16S rRNA hypervariable regions V3-V4 were amplified with indexes and

adaptor-linked universal primers (341F: 50-ACTCCTACGGGAGGCAGCAG-30, 806R: 50-GGACTACHVGGGTWTCTAA-30T). PCR was performed by using a KAPA HiFi Hotstart PCR kit (KAPA Biosystems, Wilmington, DE, USA) with high fidelity enzyme in triplicate. Amplicon libraries were quantified using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and then sequenced on the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA) for 250-bp paired-end reads. After discarding the singletons and removing chimeras, operational taxonomic units (OTUs) were generated using USEARCH (v7.0.1090) at 97% similarity by clustering the tags. Final OTUs were taxonomically classified based on the RDP classifier version 2.2 algorithm using the GreenGene database. Alpha diversity (Chao1, Shannon, Simpson) and beta diversity (principal coordinates analysis (PCoA) plots) were analyzed using QIIME version 1.7.0. In addition, a *t*-test was performed to compare the differences between groups by using STAMP. The relative abundance of bacteria is expressed as the percentage (%).

### Data analysis

The data are expressed as the mean  $\pm$  standard deviation (SD). When the data were normal and variances were equal, differences among the groups were analyzed using *t*-test. For non-normal distribution data, ln transformation was carried out before analysis. A *p*-value  $< 0.05$  was considered statistically significant. All of the statistical analyses were performed using the Statistical Package for Social Sciences version 17 software (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Body weight

The body weights of the heat-killed *S. thermophilus*-treated diabetic rats were comparable with those of the untreated diabetic rats ( $p > 0.05$ , Table 1). There was also no significant difference in body weight between the CON group and CON+ST group ( $p > 0.05$ , Table 2).

### Fasting blood glucose level and glucose tolerance

The heat-killed *S. thermophilus* treatment reduced FBG levels in diabetic rats ( $p < 0.05$ , Fig. 1). The blood glucose levels significantly decreased before and 15, 60, and 90 min after glucose load ( $p < 0.05$ , Fig. 1) in the DM+ST group as compared to those in the DM group according to the OGTT. At the time points of 30 and 120 min after the glucose load, the blood glucose levels were lower in the DM+ST group than those in DM group, but the differences were not significant ( $p > 0.05$ , Fig. 1). Compared with the DM group, the glucose AUC for the OGTT in the DM+ST group exhibited a reduced glucose AUC by 14.7% ( $p < 0.05$ , Fig. 2).

According to the OGTT, although there were no significant differences before and 15, and 120 min after the glucose load between the CON group and CON+ST group, the blood glucose levels significantly decreased 30 and 60 min after the glucose load ( $p < 0.05$ , Fig. 3) in the CON+ST group as compared to those in the CON group. The CON+ST

**Table 1** The differences in some variables between the DM+ST and DM groups.

Variables	DM	DM+ST	<i>t</i>	<i>p</i>
Weight (g)	354.2 ± 35.2	360.2 ± 33.0	-0.3	0.788
Creatinine (μmol/l)	16.0 ± 6.2	15.0 ± 2.1	0.3	0.742
ALT (U/l)	119.4 ± 51.5	123.4 ± 52.7	-0.1	0.906
Carbamide (mmol/l)	6.0 ± 0.8	7.2 ± 1.6	-1.6	0.159
Uric acid (umol/l)	116.2 ± 32.6	122.6 ± 18.2	-0.4	0.711
TC (mmol/l)	5.5 ± 0.4	4.7 ± 0.2	4.1	0.003*
Triglyceride (mmol/l)	3.0 ± 0.9	2.5 ± 0.3	1.2	0.304
HDL-C (mmol/l)	2.7 ± 0.2	2.5 ± 0.2	1.8	0.115
LDL-C (mmol/l)	1.2 ± 0.2	0.9 ± 0.1	2.5	0.063
LPS (ng/ml)	0.7 ± 0.1	0.5 ± 0.1	2.9	0.019*
LNIL6 (pg/ml)	4.6 ± 0.6	3.9 ± 0.3	2.7	0.038*
LNIL10 (pg/ml)	4.0 ± 0.3	4.5 ± 0.3	-2.4	0.046*
LnTNF-α (pg/ml)	4.1 ± 0.3	3.6 ± 0.2	3.0	0.017*
Fasting insulin (μIU/ml)	107.6 ± 18.1	67.0 ± 8.3	4.6	0.002*
HOMA-IR	106.7 ± 25.5	40.8 ± 3.8	5.7	0.004*
HbA1c (%)	12.0 ± 2.0	8.6 ± 1.2	3.3	0.011*
FBG (mmol/l)	22.3 ± 3.6	13.7 ± 1.0	3.1	0.036*

**Notes:**

*N* = 5 in each group. Data represented as means ± SD. The heat-killed *S. thermophilus* treatment reduced TC, LPS, IL-6, IL-10, TNF-α, fasting insulin levels, HbA1c, FBG, and HOMA-IR in ZDF diabetic rats.

ALT, alanine aminotransferase; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LNIL6, ln transformation of interleukin-6; LNIL10, ln transformation of interleukin-10; LnTNF-α, ln transformation of tumor necrosis factor-α; LPS, lipopolysaccharide; HOMA-IR, homeostasis model assessment of insulin resistance; FBG, fasting blood glucose.

\* *p* < 0.05.

group exhibited a reduced glucose AUC by 18.2% (*p* < 0.05, Fig. 4) for the OGTT, compared with the CON group.

### Fasting insulin, HbA1c, and HOMA-IR

The heat-killed *S. thermophilus* treatment reduced serum insulin levels, HbA1c, and HOMA-IR (*p* < 0.05, Table 1) in ZDF diabetic rats. However, compared to the CON group, the heat-killed *S. thermophilus* treatment rats failed to produce significantly lower serum insulin levels or HOMA-IR in the CON+ST group (*p* < 0.05, Table 2).

### Serum biochemical parameters

There were no significant differences in the serum creatinine, alanine aminotransferase, carbamide, or uric acid levels between the two groups in ZDF diabetic rats. The level of TC significantly increased in the DM+ST group, while the heat-killed *S. thermophilus* treatment did not significantly reduce the TG, HDL-C, or LDL-C levels in diabetic rats (*p* < 0.05, Table 1). In contrast, there were no significant difference in serum biochemical parameters in the CON+ST group compared to the CON group (*p* > 0.05, Table 1).

### Inflammatory factors

Compared with the DM group, the inflammatory factors LPS, IL-6, and TNF-α significantly decreased and IL-10 significantly increased in the DM+ST group

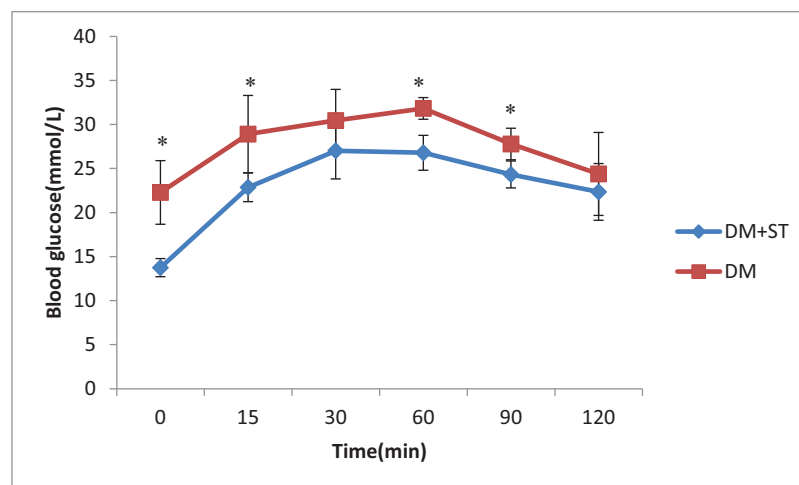
**Table 2** The differences in some variables between the CON+ST and CON groups.

Variables	CON+ST	CON	<i>t</i>	<i>p</i>
Weight (g)	503.2 ± 48.5	476.8 ± 37.3	1.0	0.363
Creatinine (μmol/l)	24.2 ± 2.8	26.2 ± 4.7	-0.8	0.437
ALT (U/l)	37.6 ± 9.8	36.8 ± 7.6	0.1	0.889
Carbamide (mmol/l)	6.1 ± 1.4	5.7 ± 1.5	0.4	0.709
Uric acid (μmol/l)	122.8 ± 21.7	154.0 ± 66.6	-1.0	0.348
TC (mmol/l)	1.4 ± 0.3	1.8 ± 0.4	-1.8	0.11
Triglyceride (mmol/l)	0.7 ± 0.2	0.7 ± 0.3	0.1	0.963
HDL-C (mmol/l)	0.8 ± 0.2	0.9 ± 0.2	-1.3	0.246
LDL-C (mmol/l)	0.3 ± 0.1	0.4 ± 0.1	-1.6	0.16
LPS (ng/ml)	0.4 ± 0.1	0.4 ± 0.1	-1.0	0.369
lnln6 (pg/ml)	4.0 ± 0.2	4.1 ± 0.3	-0.6	0.585
lnlL10 (pg/ml)	4.3 ± 0.5	4.1 ± 0.1	0.8	0.465
lnTNF-α (pg/ml)	3.9 ± 0.4	4.1 ± 0.3	-1.1	0.323
Fasting insulin (μIU/ml)	41.1 ± 7.0	42.2 ± 12.5	-0.2	0.863
FBG (mmol/l)	6.7 ± 0.6	6.9 ± 0.6	-0.4	0.72
HOMA-IR	12.3 ± 2.8	12.7 ± 2.9	-0.2	0.857

**Notes:**

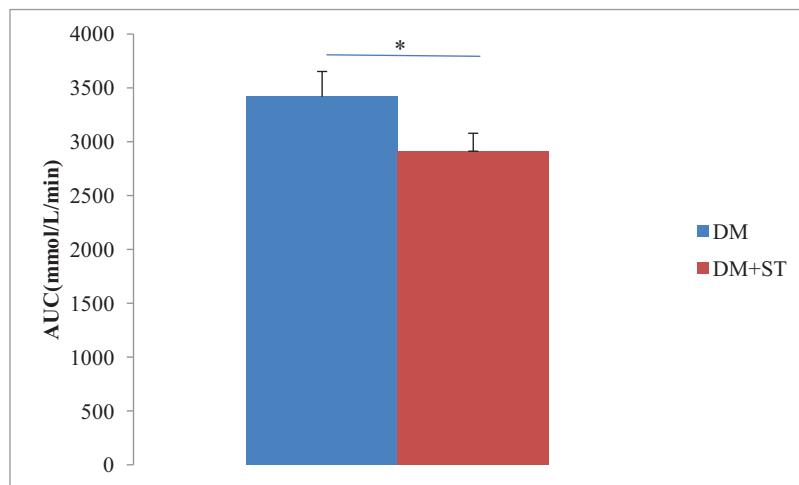
*N* = 5 in each group. Data represented as means ± SD. Compared to the CON group, the heat-killed *S. thermophilus* treatment rats failed to show significant variations.

ALT, alanine aminotransferase; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; lnln6, ln transformation of interleukin-6; lnL10, ln transformation of interleukin-10; lnTNF-α, ln transformation of tumor necrosis factor-α; LPS, lipopolysaccharide; HOMA-IR, homeostasis model assessment of insulin resistance; FBG, fasting blood glucose.

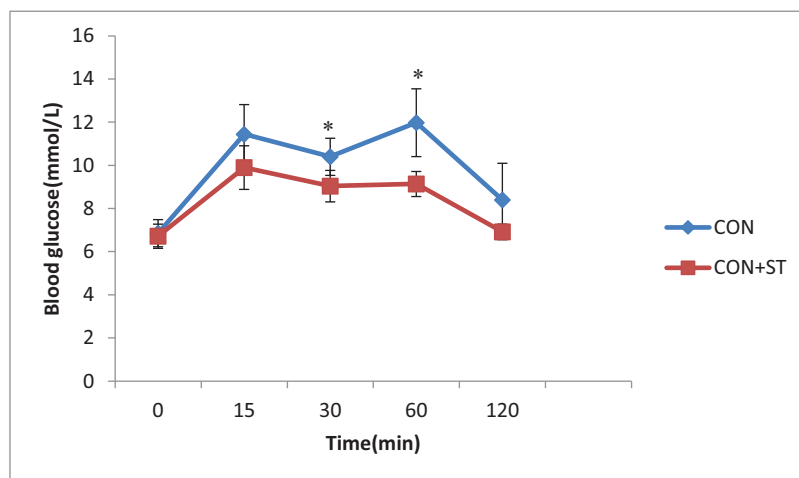


**Figure 1** The effect of heat-killed *S. thermophilus* on blood glucose during the OGTT in the DM+ST and DM groups. OGTT, oral glucose tolerance test. Error bars represent one standard deviation. The blood glucose levels significantly decreased before and 15, 60, and 90 min after glucose load in the DM+ST group as compared to those in the DM group according to the OGTT. \**p* < 0.05.

Full-size DOI: 10.7717/peerj.7117/fig-1



**Figure 2** Area under the curve (AUC) for the OGTT in the DM+ST and DM groups. Compared with the DM group, the glucose area under the curve (AUC) for the OGTT in the DM+ST group exhibited a reduced glucose AUC by 14.7%. \* $p < 0.05$ . [Full-size](#) DOI: 10.7717/peerj.7117/fig-2



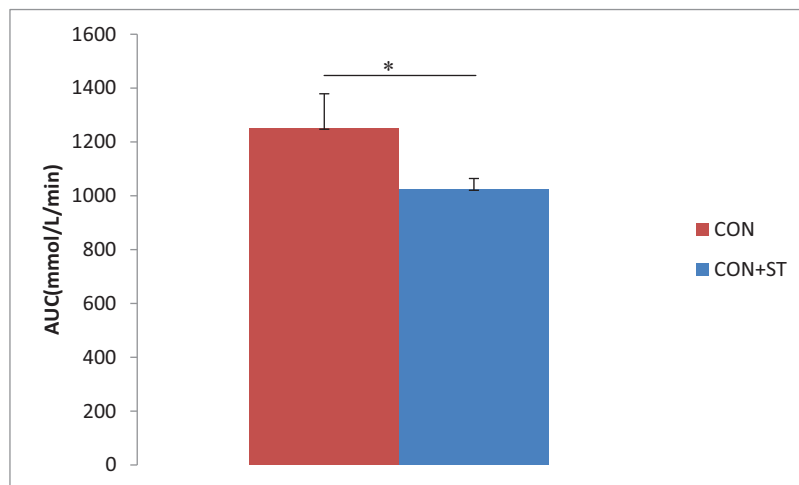
**Figure 3** The effect of heat-killed *S. thermophilus* on blood glucose during the OGTT in the CON+ST and CON groups. OGTT, oral glucose tolerance test. Error bars represent one standard deviation. According to the OGTT, although there were no significant differences before and 15, and 120 min after the glucose load between the CON group and CON+ST group, the blood glucose levels significantly decreased 30 and 60 min after the glucose load in the CON+ST group as compared to those in the CON group. \* $p < 0.05$ . [Full-size](#) DOI: 10.7717/peerj.7117/fig-3

( $p < 0.05$ , [Table 1](#)). There were no significant differences in the inflammatory factors between the CON+ST group and CON group ( $p > 0.05$ , [Table 2](#)).

### Histological analysis

We examined the heat-killed *S. thermophilus* effects on the villi length and crypt depth in the ileum. In the diabetic rats, the intestinal mucosal layer was characterized by disturbed mucosal architecture, shortened villi, blunted villus tips, and inflammatory cell infiltration. In the DM+ST group, oral administration of *S. thermophilus* restored the normal structure of the intestinal mucosal layer ([Fig. 5](#)). The length of villi and depth of crypts in the





**Figure 4** Area under the curve (AUC) for the OGTT in the CON+ST and CON groups. The CON+ST group exhibited a reduced glucose AUC by 18.2% for the OGTT, compared with the CON group. \* $p < 0.05$ . [Full-size](#) DOI: 10.7717/peerj.7117/fig-4

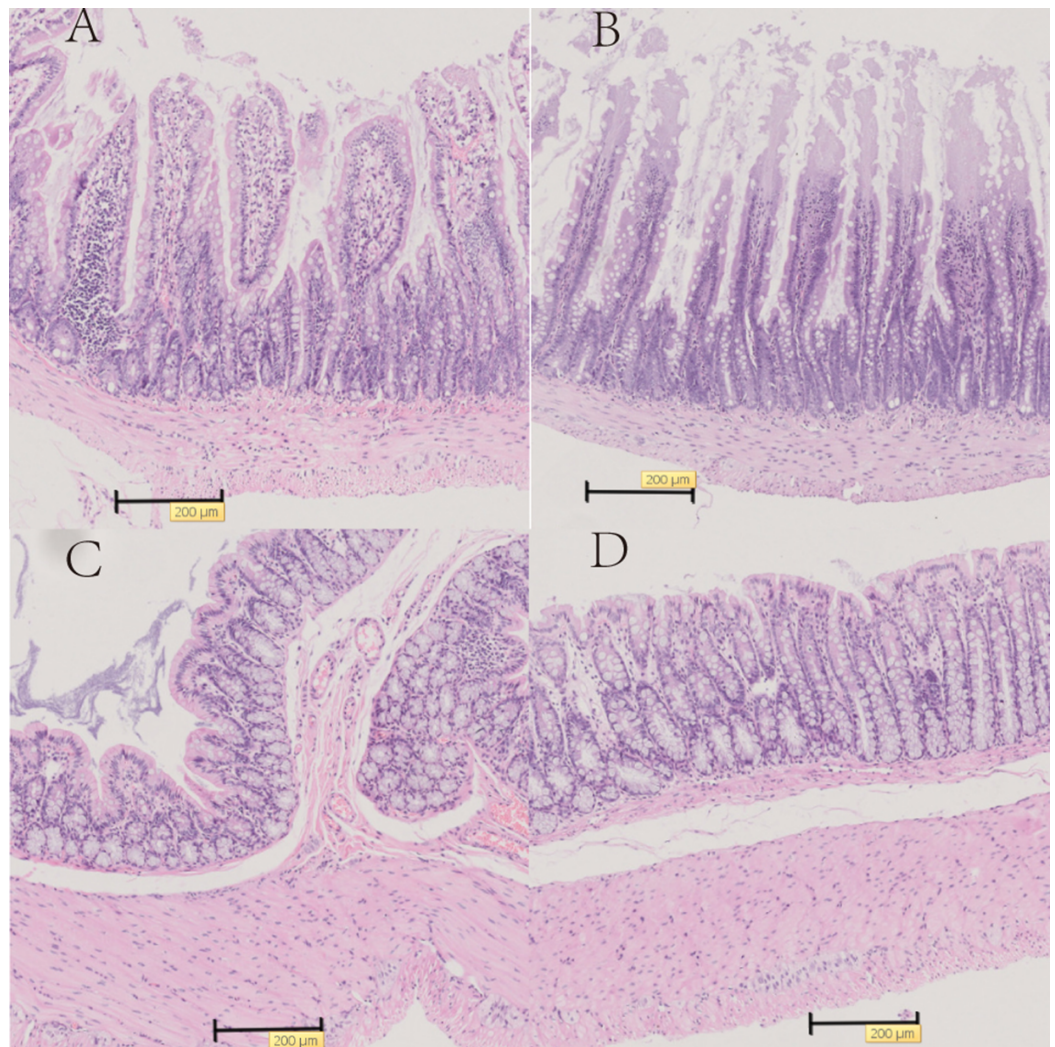
DM+ST group were significantly increased compared to those in DM group (Fig. 6). Additionally, goblet cells were counted per villus/crypt in the ileum. The ileum exhibited a significant increase in total goblet cell number after treatment with *S. thermophilus* ( $39.2 \pm 4.2$  vs  $20.9 \pm 5.0$ ,  $p < 0.05$ ). Similar findings were also seen in the colonic tissues (Figs. 5 and 7). There were no differences in the villi length and crypt depth and the numbers of goblet cells between the CON and CON+ST groups (Fig. 8).

### Western blot analysis

To explore the mechanisms underlying the heat-killed *S. thermophilus* effects on the barrier function, the expression levels of Occludin and ZO-1 proteins were determined by Western blot analysis. The results showed that Occludin and ZO-1 proteins in the DM+ST group were significantly elevated compared with the DM group both in the ileum and colon tissues (1.76-fold increases for Occludin and 2.29-fold increases for ZO-1 in the ileum tissues; 1.64-fold increases for Occludin and 1.46-fold increases for ZO-1 in the colon tissues, vs the DM group) (Fig. 9). There were no differences in the expression levels of Occludin and ZO-1 in the ileum or colon tissues between the CON and CON+ST groups (Fig. 10).

### Characterization of gut microbiota

In ZDF diabetic rats, the richness of the gut microbiota was increased in the DM+ST group compared with the DM group; however, the difference was not significant, as shown in Table 3. Significant difference did not exist between the CON+ST group and the CON group. As shown in Fig. 11, to assess the bacterial community between two groups, a PCoA for the unweighted UniFrac distance matrices was performed. The first two principal coordinates of PCoA (components 1 and 2) were separated into DM+ST and DM groups, which shared overlapping regions. As in the above analysis, the DM+ST and DM groups, and COM+ST and CON groups exhibited similar alpha and beta diversities in the gut



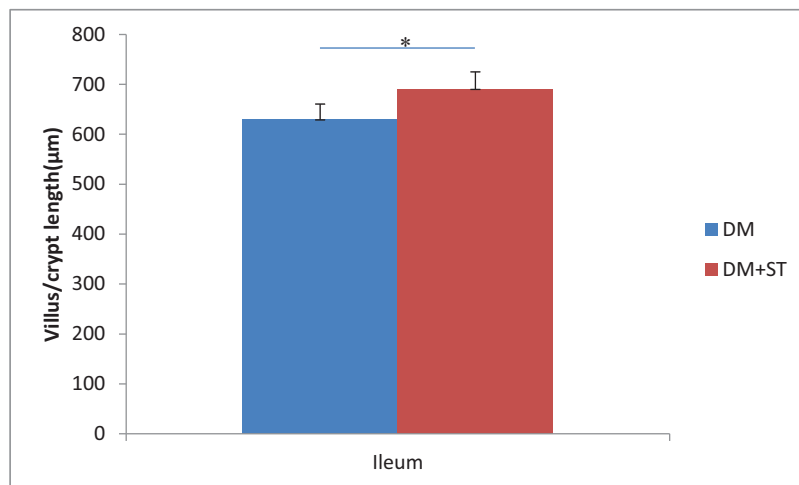
**Figure 5** Representative histology of the ileum and colon with HE stain in T2D model rats. (A) Histology of the ileum in the DM group, (B) histology of the ileum in the DM+ST group, (C) histology of the colon in the DM group, and (D) histology of the colon in the DM+ST group. The image acquisition phase was performed with a 50× objective. Scale bar = 200 µm. In the diabetic rats, the intestinal mucosal layer was characterized by disturbed mucosal architecture, shortened villi, blunted villus tips, and inflammatory cell infiltration. In the DM+ST group, oral administration of *S. thermophilus* restored the normal structure of the intestinal mucosal layer. [Full-size !\[\]\(1663bb69f307a960345edb0e712f8c02\_img.jpg\) DOI: 10.7717/peerj.7117/fig-5](https://doi.org/10.7717/peerj.7117/fig-5)

microbiota. The results indicate that the treatment with heat-killed *S. thermophilus* could not improve the richness of the gut microbiota.

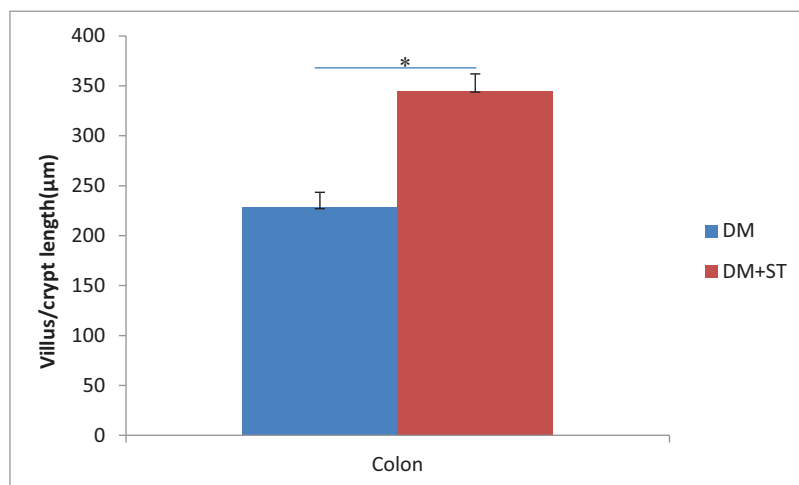
At the genus level, the abundance of *Ruminococcaceae*, *Veillonella*, *Coprococcus*, and *Bamesiella* was significantly elevated by heat-killed *S. thermophilus* treatment in ZDF diabetic rats ( $p < 0.05$ , Fig. 12), whereas *Phascolarctobacterium* and *Dorea* abundances were reduced by heat-killed *S. thermophilus* treatment in SD control rats ( $p < 0.05$ , Fig. 13).

## DISCUSSION

In this study, heat-killed *S. thermophilus* bacteria were administered to ZDF T2D rats to test whether they have a protective effect. The ZDF diabetic rat is a well-characterized

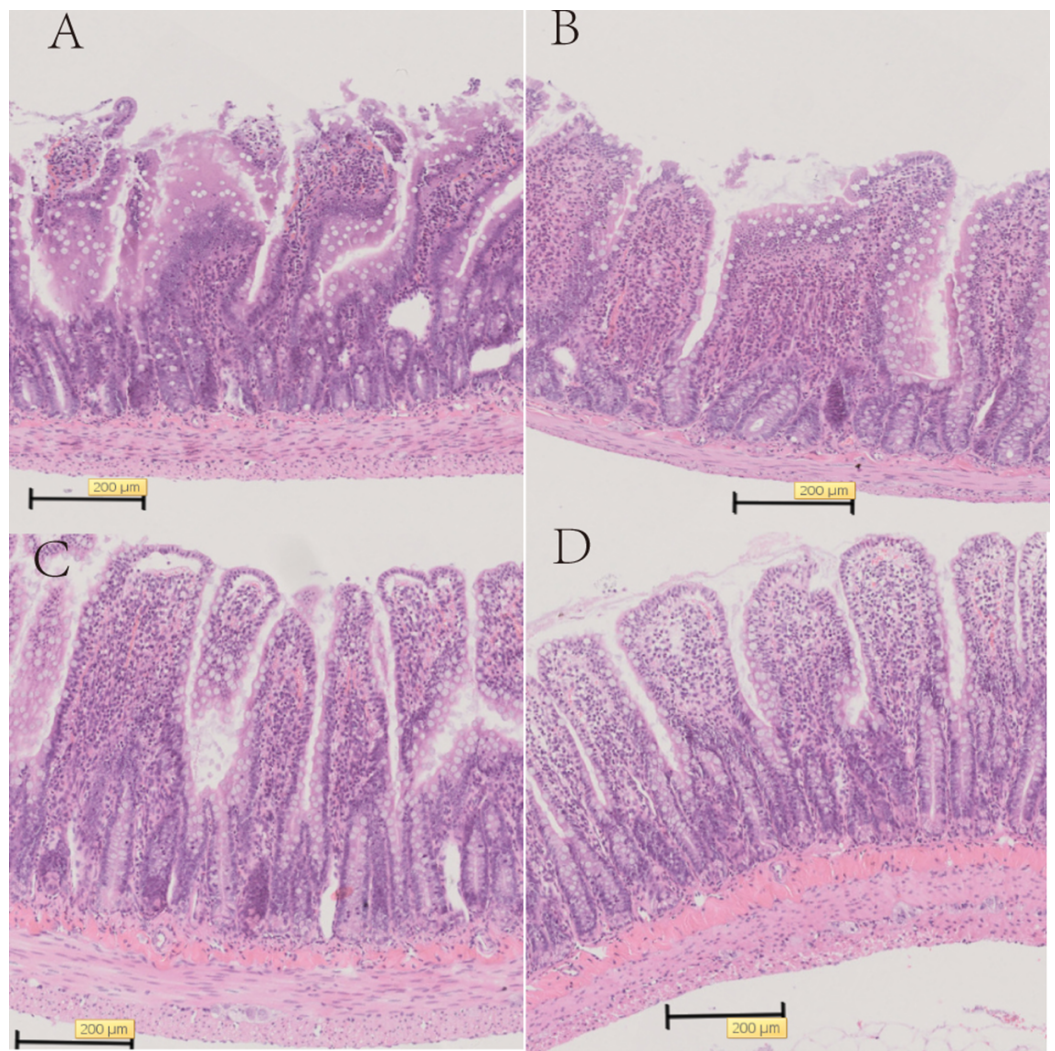


**Figure 6** The length of villi and depth of crypts in the ileum of diabetic rats. The length of villi and depth of crypts in the ileum in the DM+ST group were significantly increased compared to those in DM group. \* $p < 0.05$ . [Full-size](#) DOI: 10.7717/peerj.7117/fig-6



**Figure 7** The length of villi and depth of crypts in the colon of diabetic rats. The length of villi and depth of crypts in the colon in the DM+ST group were significantly increased compared to those in DM group. \* $p < 0.05$ . [Full-size](#) DOI: 10.7717/peerj.7117/fig-7

model of T2D, and the rats has been used in many studies to examine human T2D pathophysiology and the effects of therapeutic options (Ferreira et al., 2010; Tikellis et al., 2004). Interestingly, we found that the heat-killed *S. thermophilus* treatment effectively moderated insulin resistance and glucose intolerance in the ZDF T2D rat model. To our knowledge, this is the first report about the effect of the heat-killed *S. thermophilus* treatment on glycemic parameters of diabetic rats. Many previous studies focused on the relation between live *S. thermophilus* and human. For instance, a multispecies probiotic supplement consisting of *S. thermophilus* reduced the fasting plasma glucose and serum high-sensitivity C-reactive protein, and increased plasma total glutathione (Asemi et al., 2013b). Also, the probiotic mix VSL#3, which contains *S. thermophilus*, increased



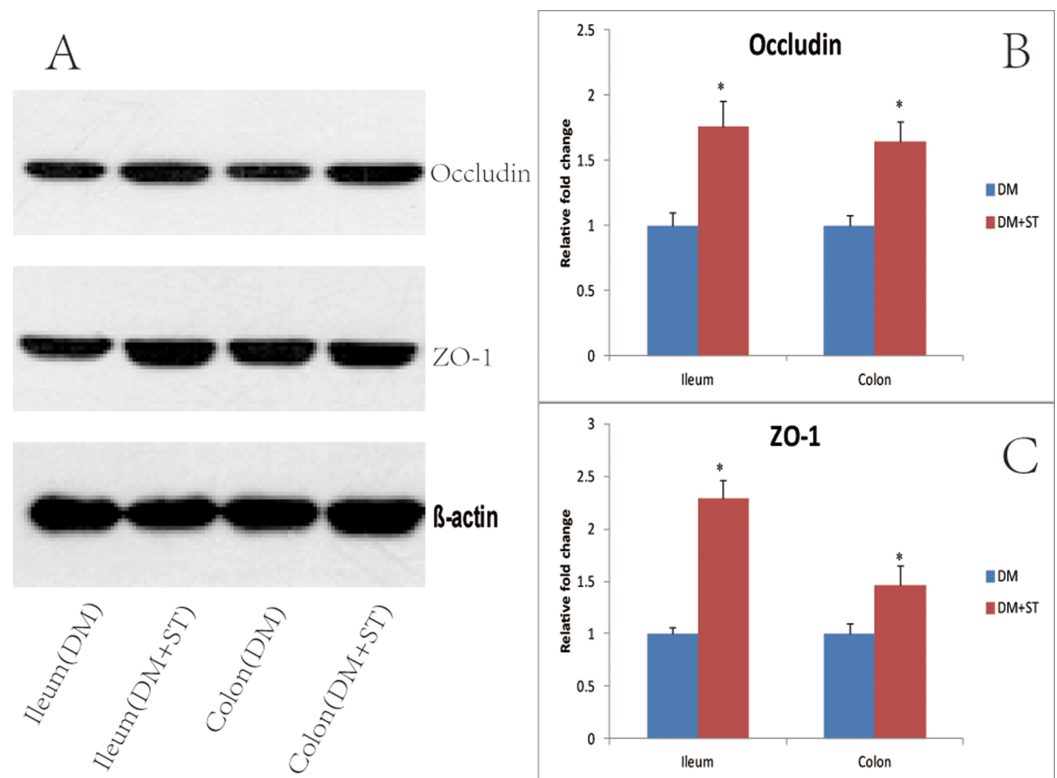
**Figure 8** Representative histology of the ileum and colon with HE stain in SD rats. (A) Histology of ileum in the CON group, (B) histology of ileum in the CON+ST group, (C) histology of colon in the CON group, (D) histology of colon in the CON+ST group. The image acquisition phase was performed with a 50× objective. Scale bar = 200 µm. The characteristics of the intestinal mucosal layer were similar between the CON+ST group and CON group. There were no differences in the villi length and crypt depth and the numbers of goblet cells between the CON+ST group and CON group.

Full-size  DOI: 10.7717/peerj.7117/fig-8

insulin sensitivity, and affected the composition of gut microbiota (*Rajkumar et al., 2014*). So our work provides new insights into the function of the heat-killed *S. thermophilus*.

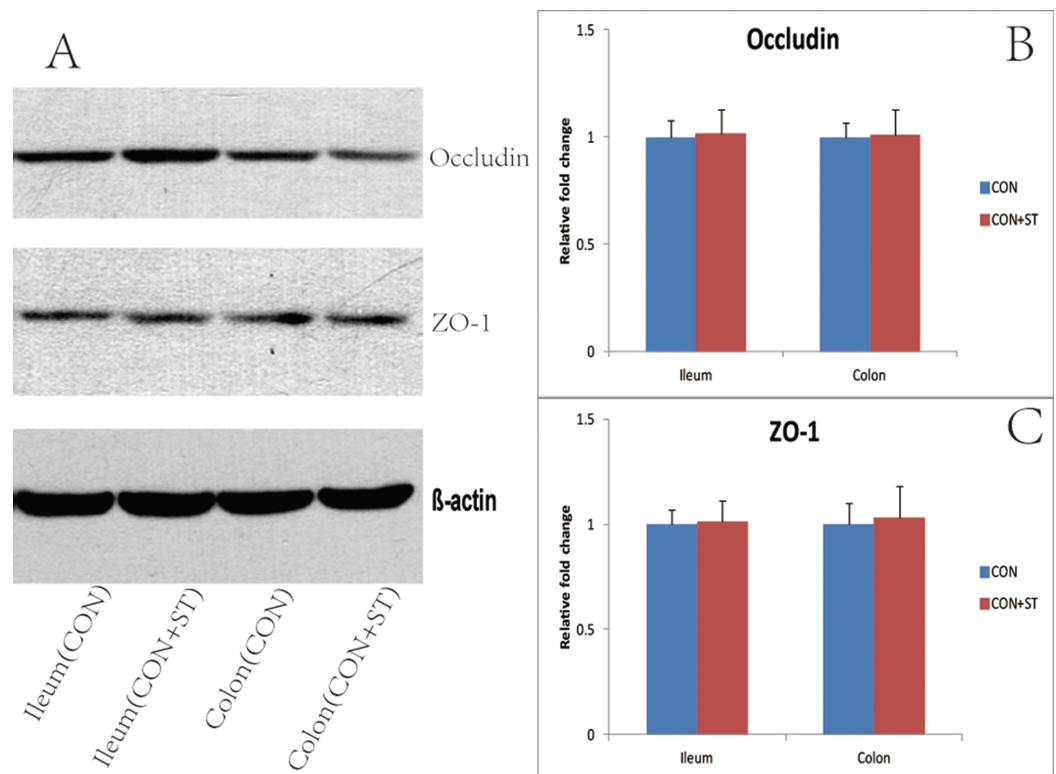
Another effect of the heat-killed *S. thermophilus* treatment is that of significantly reducing the level of TC in the diabetic rats used in this study. The effect of removing cholesterol probably occurs by two mechanisms: binding cholesterol to the cell surface (*Kimoto, Ohmomo & Okamoto, 2002; Liong & Shah, 2005*) or deconjugating bile salts to prevent their recycling (*Iyer et al., 2010; Kim et al., 2017*).

In addition, we found that the heat-killed *S. thermophilus* treatment increased the abundance of *Ruminococcaceae*, *Veillonella*, *Coprococcus*, and *Barnesiella* at the genus level



**Figure 9** Effects of the heat-killed *S. thermophilus* treatment on tight junction proteins in the DM+ST and DM groups. (A) Ileum and colon extracts from DM+ST and DM groups were used for Western blot analysis; (B) Expression levels of Occludin were quantified by measuring band densities; (C) Expression levels of ZO-1 were quantified by measuring band densities.  $\beta$ -actin was used as a loading control. \* $p < 0.05$ . [Full-size](#) DOI: 10.7717/peerj.7117/fig-9

in diabetic rats. The normal gut microbiota has many functions, such as protection against pathogens, immunomodulation, maintenance of the gut mucosal barrier structural integrity, and nutrient and drug metabolism (Jandhyala et al., 2015). As a member of short chain fatty acid producers, *Ruminococcaceae* is inversely correlated with increased intestinal permeability (Leclercq et al., 2014), and alcoholic cirrhosis (Bajaj et al., 2014). The abundance of *Ruminococcaceae* has been observed to significantly increase after treatment with fucoidan (Shang et al., 2016). *Coprococcus* is a butyrate-producing genera (Fujio-Vejar et al., 2017). Dietary intervention including extensively hydrolyzed casein formula supplemented with *Lactobacillus rhamnosus* GG to enrich *Coprococcus* could accelerate tolerance acquisition in infants who are allergic to milk (Berni et al., 2015). *Veillonella* are normal bacteria found in the intestines of mammals, that are well known for their lactate fermenting abilities. A positive association has been found between lactose levels and the abundance of the *Veillonella* genus (Pimentel et al., 2017). Anaerobic bacteria belonging to the *Barnesiella* genus enable clearance of intestinal colonization by the highly antibiotic-resistant bacterium vancomycin-resistant *Enterococcus* (Ubeda et al., 2013). When compared with high-fat, high-sucrose-fed mice, *Barnesiella* spp. are the main discriminative feature of chow-fed mice (Anhê et al., 2017). Therefore, *Barnesiella* may have a beneficial impact on host metabolism.



**Figure 10** Effects of the heat-killed *S. thermophilus* treatment on tight junction proteins in the CON+ST and CON groups. (A) Ileum and colon extracts from CON+ST and CON groups were used for Western blot analysis; (B) expression levels of Occludin were quantified by measuring band densities; (C) expression levels of ZO-1 were quantified by measuring band densities.  $\beta$ -actin was used as a loading control. [Full-size !\[\]\(1679558f37f6db0dd8360a2a7e913e90\_img.jpg\) DOI: 10.7717/peerj.7117/fig-10](https://doi.org/10.7717/peerj.7117/fig-10)

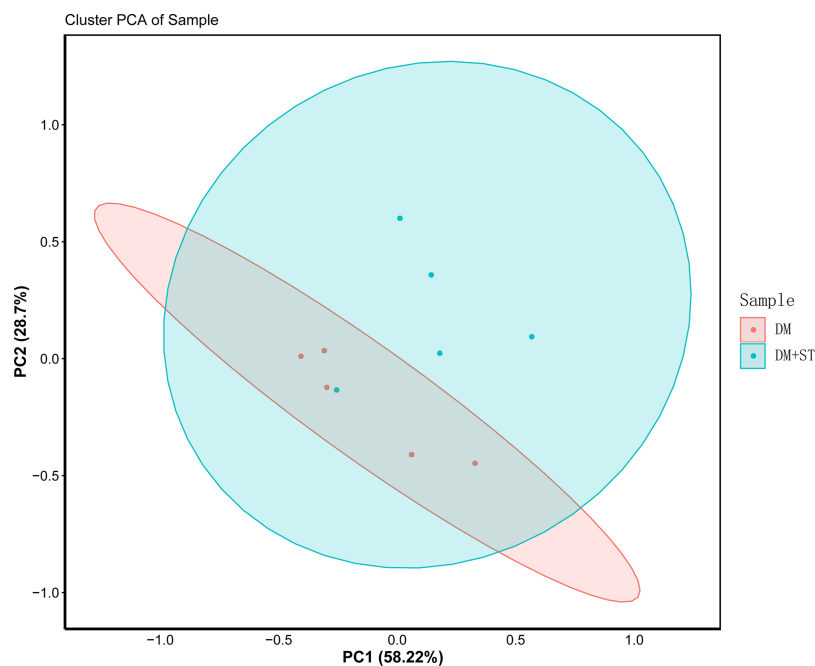
**Table 3** Alpha diversity indices.

	CON	CON+ST	DM	DM+ST
OTUs	1,497.40 $\pm$ 327.41	1,504.20 $\pm$ 275.38	1,064.60 $\pm$ 230.90	1,298.60 $\pm$ 323.53
Chao1	4,143.66 $\pm$ 490.57	4,218.70 $\pm$ 524.60	3,253.11 $\pm$ 518.16	3,185.49 $\pm$ 733.41
Shannon	117.34 $\pm$ 11.09	121.01 $\pm$ 11.65	86.96 $\pm$ 8.13	90.94 $\pm$ 20.40
Simpson	6.76 $\pm$ 0.66	6.88 $\pm$ 0.39	4.92 $\pm$ 0.70	5.72 $\pm$ 1.22
PD_whole_tree	0.93 $\pm$ 0.04	0.93 $\pm$ 0.02	0.81 $\pm$ 0.08	0.87 $\pm$ 0.09

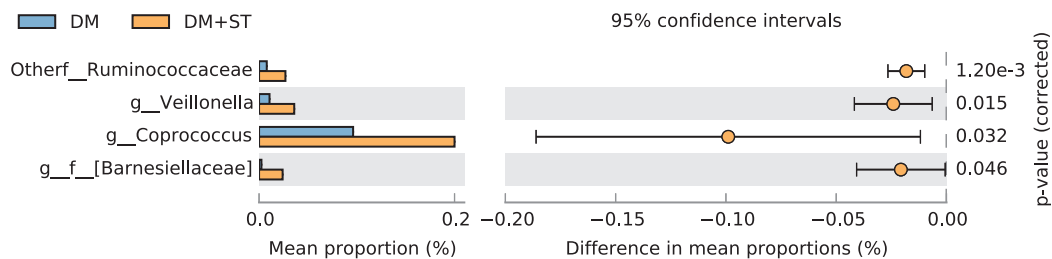
**Note:**

Data are presented as means  $\pm$  SD ( $n = 5$ ). In ZDF diabetic rats, the richness of the gut microbiota was increased in the DM+ST group compared with the DM group; however, the difference was not significant ( $p > 0.05$ ,  $t$ -test and Wilcoxon rank-sum test). Significant difference did not exist between the CON+ST group and the CON group ( $p > 0.05$ ,  $t$ -test and Wilcoxon rank-sum test).

Many effects of probiotics are mediated through immune regulation and through the balance of anti-inflammatory and pro-inflammatory cytokines. In this study, the heat-killed *S. thermophilus* treatment significantly decreased the inflammatory factors LPS, IL-6, and TNF- $\alpha$ , and increased IL-10. From the membranes of gram-negative bacteria, LPS penetrates into the blood via impaired permeability of the intestinal mucosa, which is caused by the reduced expression of adhesion and tight junction proteins (Cani *et al.*, 2008). Then, LPS triggers a strong pro-inflammatory reaction and secretion of

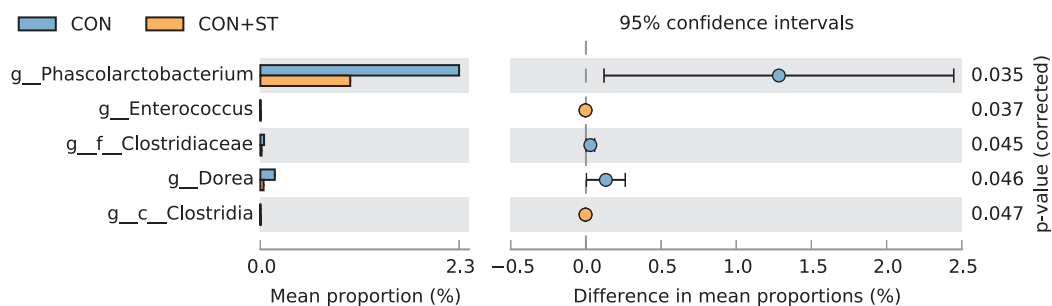


**Figure 11** PCoA of unweighted UniFrac distances of the gut bacterial communities between the DM+ST and DM groups. The first two principal coordinates of PCoA were separated into DM+ST and DM groups, which shared overlapping regions. [Full-size !\[\]\(fd7fe780e8fd8eece60268c87d0c3e04\_img.jpg\) DOI: 10.7717/peerj.7117/fig-11](https://doi.org/10.7717/peerj.7117/fig-11)



**Figure 12** The *t*-test results of the relative abundance (%) of bacteria from the DM+ST and DM groups. At the genus level, the abundance of *Ruminococcaceae*, *Veillonella*, *Coprococcus*, and *Barnesiella* was significantly elevated by heat-killed *S. thermophilus* treatment in ZDF diabetic rats. [Full-size !\[\]\(86257f54800c9844bc7e863bea396fba\_img.jpg\) DOI: 10.7717/peerj.7117/fig-12](https://doi.org/10.7717/peerj.7117/fig-12)

proinflammatory cytokines from the host cells, followed by metabolic endotoxemia (Bäckhed *et al.*, 2003). Metabolic endotoxemia increases systemic inflammation and impairs insulin sensitivity in both adipose tissue and the liver (Cani *et al.*, 2007). It can also impair insulin signaling by inducing endoplasmic reticulum stress and the activity of a histone acetyltransferase (Cao *et al.*, 2017). The high circulating LPS characterizes both incident and prevalent diabetes in a clinical observation also suggests the relevance of this putative mechanism to humans (Pussinen *et al.*, 2011). As the product of pro-inflammatory cells, IL-6 is involved in many biological processes, such as the host response to acute-phase reactions, hematopoiesis, enteric pathogens, and terminal differentiation of B-lymphocytes (Adams, 2010). IL-10 is a potent deactivator of



**Figure 13** The *t*-test results of the relative abundance (%) of bacteria from the CON+ST and CON groups. Compared with the CON group, *Phascolarctobacterium* and *Dorea* abundances were reduced by heat-killed *S. thermophilus* treatment in the CON+ST group.

Full-size DOI: [10.7717/peerj.7117/fig-13](https://doi.org/10.7717/peerj.7117/fig-13)

macrophage/monocyte proinflammatory cytokine synthesis (Clarke *et al.*, 2015), such as downregulation of TNF- $\alpha$  secretion by macrophages (Fiorentino *et al.*, 1991).

It was also found that the heat-killed *S. thermophilus* treatment protected the intestinal barrier. In our study, an increased ileum villus/crypt length and number of goblet cells were observed in the DM+ST group with *S. thermophilus* administration compared with the DM group. This is consistent with previous studies reporting that probiotic administration markedly deepened jejunal crypts in healthy rats (Tazuke *et al.*, 2011), and both villus and crypt were lengthened after treatment by emu oil (Abimosleh *et al.*, 2012). The main role of goblet cells is to protect the mucous membrane by secreting mucus (Robbe-Masselot *et al.*, 2004). There is a strong association between intestinal flora and secretion of mucin (Yeung *et al.*, 2015), as goblet cells may be regulated by interactions between the gastrointestinal mucosa and specific bacterial peptides (Leiper *et al.*, 2001). The results of our study also showed that the Occludin and ZO-1 proteins in the DM+ST group were significantly elevated compared with the DM group both in the ileum and colon tissues. Intestinal barrier integrity is maintained by the tight junctions those are made of transmembrane, scaffold and adaptor proteins. Occludin is transmembrane protein embedded in the intracellular actin through attachment to adaptor protein ZO-1 (Bauer *et al.*, 2010). It is widely reported that commensal bacteria have profound effects on epithelial integrity and permeability, particularly, on tight junctions maintenance (Alam & Neish, 2018). A dysbiosis adversely enhances intestinal permeability by modulating the expression of epithelial tight junction proteins ZO-1 and Occludin (Cani *et al.*, 2009). The mucosal barrier is very important for protecting the host tissue from damage that is mediated by toxic products or luminal pathogens obtained from food or pathogenic bacteria, while allowing uptake of nutrients at the same time. A previous study showed that feeding fermented milk produced by *S. thermophilus* and *Bifidobacterium breve* could reinforce the intestinal barrier (Terpend *et al.*, 1999). Another study also showed that live *S. thermophilus* significantly increased the transepithelial electrical resistance in the intestinal Caco-2 cell monolayer by enhancement (actinin, occludin) or maintenance (actin, ZO-1) of cytoskeletal and tight junctional protein phosphorylation (Resta-Lenert & Barrett, 2003).



In the current study, the *S. thermophilus* used was heat-killed instead of live cells. Both live and dead cells are capable of generating a biological response (Dotan & Rachmilewitz, 2005). Our result is consistent with a recent study which shows that pasteurized *Akkermansia muciniphila* is able to ameliorate high-fat diet induced dysglycemia (Plovier et al., 2017). In a meta-analysis, modified (heat-killed or sonicated) probiotics were found to have effects similar to those of the living probiotics in most trials (Zorzela et al., 2017). The effects of heat-killed probiotics may be attributed to the dead cells and/or their metabolites. For example, metabolites released by *S. thermophilus* exerted an anti-TNF- $\alpha$  effect and were capable of crossing the intestinal barrier (Ménard et al., 2004). Besides, a recombinant protein isolated from the *A. muciniphila* membrane can lead to an improved gut barrier (Plovier et al., 2017). Notably, even *A. muciniphila*-derived extracellular vesicles can decrease gut permeability by regulating the tight junctions (Chelakkot et al., 2018). It has also been documented that bacterial muramyl dipeptide reduces inflammation and promotes insulin signaling in the state of metabolic endotoxemia, and glycemia (Cavallari et al., 2017). As a bacterial metabolite, indole is able to counteract the pro-inflammatory and metabolism-altering effects of LPS in the liver (Beaumont et al., 2018). Similarly, SCFAs can improve barrier function (Elamin et al., 2013), decrease inflammation, and promote the metabolism of lipids and glucose (Sonnenburg & Bäckhed, 2016; Canfora, Jocken & Blaak, 2015). In addition, microbiota-derived succinate can also improve glucose metabolism by acting on intestinal gluconeogenesis (De Vadder et al., 2016).

One limitation to widespread use of probiotic therapy is the concern regarding adverse effects, which may cause some pathology of their own (Berger, 2005). Compared with live probiotics, heat-killed probiotics are safer for purposes such as application in immunosuppressed patients and children (Vintiñi & Medina, 2011). Another problem with live probiotics is that they would have to survive proteolytic enzymes and the low pH of stomach acid. The recovery rate of total *S. thermophilus* from the terminal ileum of minipigs was very low after digesting a certain amount of live cells (Lick, Drescher & Heller, 2001). The preparation and administration of heat-killed probiotics are convenient compared to live probiotics (Josef & Ricardo, 2005). Products based on dead cells are easier to standardize, and store, and they also have a long shelf-life (Adams, 2010). Therefore, heat-killed probiotics may be a promising and safer alternative to live probiotics.

After the analysis of numerous studies, it was proposed that there may be a bacteria mucosal immunity-inflammation-diabetes (BMID) axis, through which herbal monomers and formulae improve diabetes (Gao et al., 2017). In this study, heat-killed *S. thermophilus* may also affect diabetes through the BMID axis by increasing the abundance of beneficial bacteria, protecting the intestinal epithelial barrier, and suppressing IL-6, LPS, and TNF- $\alpha$  secretion, and the end result is moderation of insulin tolerance.

## CONCLUSION

Our study supports the hypothesis that treatment with heat-killed *S. thermophilus* could effectively improve the glycemic parameters of T2D model rats. In addition, the potential mechanisms underlying the protection may consist of changing the

composition of gut microbiota, reinforcing the intestinal epithelial barrier and the immunity of the intestinal mucosa, decreasing the level of inflammation, and then reducing insulin resistance.

## ACKNOWLEDGEMENTS

We are very grateful to CapitalBio Technology Co., Ltd. for excellent technical assistance with 16s sequencing experiments, and thank LetPub for its linguistic assistance during the preparation of this manuscript.

## ADDITIONAL INFORMATION AND DECLARATIONS

### Funding

This work was supported by Australia-China International Collaborative Grant (NH&MRC-APP1112767-NSFC81561128020-0023) and National Natural Science Foundation of China (81872920). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Grant Disclosures

The following grant information was disclosed by the authors:

Australia-China International Collaborative Grant: NH&MRC-APP1112767-NSFC81561128020-0023.

National Natural Science Foundation of China: 81872920.

### Competing Interests

The authors declare that they have no competing interests.

### Author Contributions

- Xiangyang Gao conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Fei Wang conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Peng Zhao contributed reagents/materials/analysis tools, approved the final draft.
- Rong Zhang contributed reagents/materials/analysis tools, approved the final draft.
- Qiang Zeng conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

### Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

All experimental protocols were approved by the Animal Care Committee of the General PLA Hospital Animal Ethics Committee (project: CPLAGHAE-20171228-01).

## Data Availability

The following information was supplied regarding data availability:

The raw data is available in the [Supplemental Files](#) and at Zenodo: Xiangyang Gao. (2019). Effect of heat-killed *Streptococcus thermophilus* on type 2 diabetes rats [Data set]. Zenodo. DOI [10.5281/zenodo.2553135](https://doi.org/10.5281/zenodo.2553135).

## Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.7117#supplemental-information>.

## REFERENCES

- Abimosleh SM, Lindsay RJ, Butler RN, Cummins AG, Howarth GS. 2012.** Emu oil increases colonic crypt depth in a rat model of ulcerative colitis. *Digestive Diseases and Sciences* **57**(4):887–896 DOI [10.1007/s10620-011-1979-1](https://doi.org/10.1007/s10620-011-1979-1).
- Adams CA. 2010.** The probiotic paradox: live and dead cells are biological response modifiers. *Nutrition Research Reviews* **23**(1):37–46 DOI [10.1017/s0954422410000090](https://doi.org/10.1017/s0954422410000090).
- Alam A, Neish A. 2018.** Role of gut microbiota in intestinal wound healing and barrier function. *Tissue Barriers* **6**(3):1539595 DOI [10.1080/21688370.2018.1539595](https://doi.org/10.1080/21688370.2018.1539595).
- Amandine E, Clara B, Lucie G, Ouwerkerk JP, Céline D, Bindels LB, Yves G, Muriel D, Muccioli GG, Delzenne NM. 2013.** Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences of the United States of America* **110**(22):9066–9071 DOI [10.1073/pnas.1219451110](https://doi.org/10.1073/pnas.1219451110).
- Anhê FF, Nachbar RT, Varin TV, Vilela V, Dudonné S, Pilon G, Fournier M, Lecours MA, Desjardins Y, Roy D, Levy E, Marette A. 2017.** A polyphenol-rich cranberry extract reverses insulin resistance and hepatic steatosis independently of body weight loss. *Molecular Metabolism* **6**(12):1563–1573 DOI [10.1016/j.molmet.2017.10.003](https://doi.org/10.1016/j.molmet.2017.10.003).
- Asemi Z, Samimi M, Tabassi Z, Rad MN, Foroushani AR, Khorammian H, Esmailzadeh A. 2013.** Effect of daily consumption of probiotic yoghurt on insulin resistance in pregnant women: a randomized controlled trial. *European Journal of Clinical Nutrition* **67**(1):71–74 DOI [10.1038/ejcn.2012.189](https://doi.org/10.1038/ejcn.2012.189).
- Asemi Z, Zare Z, Shakeri H, Sabihi SS, Esmailzadeh A. 2013.** Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes. *Annals of Nutrition and Metabolism* **63**(1-2):1–9 DOI [10.1159/000349922](https://doi.org/10.1159/000349922).
- Bäckhed F, Normark S, Schweda EKH, Oscarson S, Richter-Dahlfors A. 2003.** Structural requirements for TLR4-mediated LPS signalling: a biological role for LPS modifications. *Microbes and Infection* **5**(12):1057–1063 DOI [10.1016/S1286-4579\(03\)00207-7](https://doi.org/10.1016/S1286-4579(03)00207-7).
- Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. 2014.** Altered profile of human gut microbiome is associated with cirrhosis and its complications. *Journal of Hepatology* **60**(5):940–947 DOI [10.1016/j.jhep.2013.12.019](https://doi.org/10.1016/j.jhep.2013.12.019).
- Baothman OA, Zamzami MA, Taher I, Abubaker J, Abu-Farha M. 2016.** The role of gut microbiota in the development of obesity and diabetes. *Lipids in Health & Disease* **15**(1):108 DOI [10.1186/s12944-016-0278-4](https://doi.org/10.1186/s12944-016-0278-4).
- Bauer H, Zweimueller-Mayer J, Steinbacher P, Lametschwandtner A, Bauer HC. 2010.** The dual role of zonula occludens (ZO) proteins. *Journal of Biomedicine & Biotechnology* **2010**:402593 DOI [10.1155/2010/402593](https://doi.org/10.1155/2010/402593).

- Beaumont M, Neyrinck AM, Olivares M, Rodriguez J, De Rocca Serra A, Roumain M, Bindels LB, Cani PD, Evenepoel P, Muccioli GG, Demoulin J-B, Delzenne NM. 2018. The gut microbiota metabolite indole alleviates liver inflammation in mice. *Faseb Journal* 32(12):6681–6693 DOI 10.1096/fj.201800544.
- Berger RE. 2005. Lactobacillus sepsis associated with probiotic therapy. *Journal of Urology* 174(5):1843–1843 DOI 10.1016/s0022-5347(01)68804-9.
- Berni CR, Sangwan N, Stefka AT, Nocerino R, Paparo L, Aitoro R, Calignano A, Khan AA, Jack A, Gilbert JA, Nagler CR. 2015. *Lactobacillus rhamnosus* GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *ISME Journal* 10(3):742–750 DOI 10.1038/ismej.2015.151.
- Boulangé C, Neves AL, Chilloux J, Nicholson J, Dumas ME. 2016. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Medicine* 8(1):42 DOI 10.1186/s13073-016-0303-2.
- Bruno-Barcelona JM, Andrus JM, Libby SL, Klaenhammer TR, Hassan HM. 2004. Expression of a heterologous manganese superoxide dismutase gene in intestinal lactobacilli provides protection against hydrogen peroxide toxicity. *Applied & Environmental Microbiology* 70(8):4702–4710 DOI 10.1128/AEM.70.8.4702-4710.2004.
- Canfora EE, Jocken JW, Blaak EE. 2015. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nature Reviews Endocrinology* 11(10):577–591 DOI 10.1038/nrendo.2015.128.
- Cani P, Amar J, Iglesias M, Poggi M, Knauf C, Bastelica D, Neyrinck A, Fava F, Tuohy K, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti J-F, Gibson GR, Casteilla L, Burcelin R. 2007. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56(7):1761–1772 DOI 10.2337/db06-1491.
- Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 57(6):1470–1481 DOI 10.2337/db07-1403.
- Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM. 2009. Changes in gut microbiota control inflammation in obese mice through a mechanism involving glp-2-driven improvement of gut permeability. *Gut* 58(8):1091–1103 DOI 10.1136/gut.2008.165886.
- Cao J, Peng J, An H, He Q, Boronina T, Guo S, White MF, Cole PA, He L. 2017. Endotoxemia-mediated activation of acetyltransferase P300 impairs insulin signaling in obesity. *Nature Communications* 8(1):131 DOI 10.1038/s41467-017-00163-w.
- Cavallari JF, Fullerton MD, Duggan BM, Foley KP, Denou E, Smith BK, Desjardins EM, Henriksbo BD, Kim KJ, Tuinema BR, Stearns JC, Prescott D, Rosenstiel P, Coombes BK, Steinberg GR, Schertzer JD. 2017. Muramyl dipeptide-based postbiotics mitigate obesity-induced insulin resistance via IRF4. *Cell Metabolism* 25(5):1063–1074.e3 DOI 10.1016/j.cmet.2017.03.021.
- Chelakkot C, Choi Y, Kim D-K, Park HT, Ghim J, Kwon Y, Jeon J, Kim M-S, Jee Y-K, Gho YS, Park H-S, Kim Y-K, Ryu SH. 2018. *Akkermansia muciniphila*-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. *Experimental & Molecular Medicine* 50(2):e450 DOI 10.1038/emm.2017.282.
- Clarke CJP, Hales A, Hunt A, Foxwell BMJ. 2015. IL-10-mediated suppression of TNF- $\alpha$  production is independent of its ability to inhibit NF $\kappa$ B activity. *European Journal of Immunology* 28(5):1719–1726 DOI 10.1002/(sici)1521-4141(199805)28:05<1719::aid-immu1719>3.0.co;2-q.

- De Vadder F, Kovatcheva-Datchary P, Zitoun C, Duchamp A, Bäckhed F, Mithieux G. 2016.** Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. *Cell Metabolism* **24**(1):151–157 DOI [10.1016/j.cmet.2016.06.013](https://doi.org/10.1016/j.cmet.2016.06.013).
- Delorme C. 2008.** Safety assessment of dairy microorganisms: *Streptococcus thermophilus*. *International Journal of Food Microbiology* **126**(3):274–277 DOI [10.1016/j.ijfoodmicro.2007.08.014](https://doi.org/10.1016/j.ijfoodmicro.2007.08.014).
- Donkor ON, Ravikumar M, Proudfoot O, Day SL, Apostolopoulos V, Paukovics G, Vasiljevic T, Nutt SL, Gill H. 2012.** Cytokine profile and induction of T helper type 17 and regulatory T cells by human peripheral mononuclear cells after microbial exposure. *Clinical & Experimental Immunology* **167**(2):282–295 DOI [10.1111/j.1365-2249.2011.04496.x](https://doi.org/10.1111/j.1365-2249.2011.04496.x).
- Dotan I, Rachmilewitz D. 2005.** Probiotics in inflammatory bowel disease: possible mechanisms of action. *Current Opinion in Gastroenterology* **21**(4):426–430.
- Ehud B, Felix M, Marisa H, Abraham W. 2004.** Lactobacillus GG bacteria ameliorate arthritis in Lewis rats. *Journal of Nutrition* **135**(7):1752–1756.
- Elamin EE, Masclee AA, Dekker J, Pieters H-J, Jonkers DM. 2013.** Short-chain fatty acids activate AMP-activated protein kinase and ameliorate ethanol-induced intestinal barrier dysfunction in caco-2 cell monolayers. *Journal of Nutrition* **143**(12):1872–1881 DOI [10.3945/jn.113.179549](https://doi.org/10.3945/jn.113.179549).
- FAO/WHO. 2001.** Report on joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Available at <http://www.fao.org/es/ESN/Probio/probio.htm>.
- Ferreira L, Teixeira-De-Lemos E, Pinto F, Parada B, Mega C, Vala H, Pinto R, Garrido P, Sereno J, Fernandes R, Santos P, Velada I, Melo A, Nunes S, Teixeira F, Reis F. 2010.** Effects of sitagliptin treatment on dysmetabolism, inflammation, and oxidative stress in an animal model of type 2 diabetes (ZDF rat). *Mediators of Inflammation* **2010**: Article ID 59276011.
- Finegood D, McArthur MD, Kojwang D, Thomas MJ, Topp BG, Leonard T, Buckingham RE. 2001.** Beta-cell mass dynamics in Zucker diabetic fatty rats: rosiglitazone prevents the rise in net cell death. *Diabetes* **50**(5):1021–1029 DOI [10.2337/diabetes.50.5.1021](https://doi.org/10.2337/diabetes.50.5.1021).
- Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O’Garra A. 1991.** IL-10 inhibits cytokine production by activated macrophages. *Journal of Immunology* **147**(11):3815–3822.
- Fujio-vejar S, Vasquez Y, Morales P, Magne F, Vera-Wolf P, Ugalde JA, Navarrete P, Gotteland M. 2017.** The Gut Microbiota of Healthy Chilean Subjects Reveals a High Abundance of the Phylum Verrucomicrobia. *Frontiers in Microbiology* **8**:1221 DOI [10.3389/fmicb.2017.01221](https://doi.org/10.3389/fmicb.2017.01221).
- Gao Z, Li Q, Wu X, Zhao X, Zhao L, Tong X. 2017.** New insights into the mechanisms of chinese herbal products on diabetes: a focus on the “Bacteria-Mucosal Immunity-Inflammation-Diabetes” axis. *Journal of Immunology Research* **2017**:1–13 DOI [10.1155/2017/1813086](https://doi.org/10.1155/2017/1813086).
- Haro C, Montes-Borrego M, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P, Delgado-Lista J, Quintana-Navarro GM, Tinahones FJ, Landa BB. 2015.** Two healthy diets modulate gut microbial community improving insulin sensitivity in a human obese population. *Journal of Clinical Endocrinology & Metabolism* **101**(1):jc20153351 DOI [10.1210/jc.2015-3351](https://doi.org/10.1210/jc.2015-3351).
- Hartstra AV, Nieuwdorp M, Herrema H. 2016.** Interplay between gut microbiota, its metabolites and human metabolism: dissecting cause from consequence. *Trends in Food Science & Technology* **57**:233–243 DOI [10.1016/j.tifs.2016.08.009](https://doi.org/10.1016/j.tifs.2016.08.009).
- Iyer R, Tomar SK, Maheswari TU, Singh R. 2010.** *Streptococcus thermophilus* strains: multifunctional lactic acid bacteria. *International Dairy Journal* **20**(3):133–141 DOI [10.1016/j.idairyj.2009.10.005](https://doi.org/10.1016/j.idairyj.2009.10.005).

- Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. 2015. Role of the normal gut microbiota. *World Journal of Gastroenterology* **21**(29):8787–8803 DOI [10.3748/wjg.v21.i29.8787](https://doi.org/10.3748/wjg.v21.i29.8787).
- Jorge HM, Eran E, Chengcheng J, Liming H, Mehal WZ, Till S, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ. 2012. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* **482**(7384):179–185.
- Josef N, Ricardo C. 2005. Probiotics: protecting the intestinal ecosystem? *Journal of Pediatrics* **147**(2):143–146 DOI [10.1016/j.jpeds.2005.05.033](https://doi.org/10.1016/j.jpeds.2005.05.033).
- Kilic AO, Pavlova SI, Ma WG, Tao L. 1996. Analysis of Lactobacillus phages and bacteriocins in American dairy products and characterization of a phage isolated from yogurt. *Applied & Environmental Microbiology* **62**(6):2111–2116.
- Kim SJ, Park SH, Sin HS, Jang SH, Lee SW, Kim SY, Kwon B, Yu KY, Kim SY, Yang DK. 2017. Hypocholesterolemic effects of probiotic mixture on diet-induced hypercholesterolemic rats. *Nutrients* **9**(3):E293 DOI [10.3390/nu9030293](https://doi.org/10.3390/nu9030293).
- Kimoto H, Ohmomo S, Okamoto T. 2002. Cholesterol removal from media by lactococci. *Journal of Dairy Science* **85**(12):3182–3188 DOI [10.3168/jds.S0022-0302\(02\)74406-8](https://doi.org/10.3168/jds.S0022-0302(02)74406-8).
- Larsen N, Vogensen FK, Van Den Berg FWJ, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. 2010. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLOS ONE* **5**(2):e9085 DOI [10.1371/journal.pone.0009085](https://doi.org/10.1371/journal.pone.0009085).
- Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Starkel P, Windey K, Tremaroli V, Backhed F, Verbeke K, De Timary P, Delzenne NM. 2014. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proceedings of the National Academy of Sciences of the United States of America* **111**(42):E4485–E4493 DOI [10.1073/pnas.1415174111](https://doi.org/10.1073/pnas.1415174111).
- Leiper K, Campbell BJ, Jenkinson MD, Milton J, Yu L-G, Democratis J, Rhodes JM. 2001. Interaction between bacterial peptides, neutrophils and goblet cells: a possible mechanism for neutrophil recruitment and goblet cell depletion in colitis. *Clinical Science* **101**(4):395–402 DOI [10.1042/cs1010395](https://doi.org/10.1042/cs1010395).
- Leonard BL, Watson RN, Loomes KM, Phillips ARJ, Cooper GJ. 2005. Insulin resistance in the Zucker diabetic fatty rat: a metabolic characterisation of obese and lean phenotypes. *Acta Diabetologica* **42**(4):162–170 DOI [10.1007/s00592-005-0197-8](https://doi.org/10.1007/s00592-005-0197-8).
- Li C, Li X, Han H, Cui H, Peng M, Wang G, Wang Z. 2016. Effect of probiotics on metabolic profiles in type 2 diabetes mellitus: a meta-analysis of randomized, controlled trials. *Medicine* **95**(26):e4088 DOI [10.1097/MD.0000000000004088](https://doi.org/10.1097/MD.0000000000004088).
- Lick S, Drescher K, Heller KJ. 2001. Survival of *Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus* in the terminal ileum of fistulated Gottingen minipigs. *Applied and Environmental Microbiology* **67**(9):4137–4143 DOI [10.1128/aem.67.9.4137-4143.2001](https://doi.org/10.1128/aem.67.9.4137-4143.2001).
- Lin MY, Yen CL. 1999. Antioxidative ability of lactic acid bacteria. *Journal of Agricultural and Food Chemistry* **47**(4):1460–1466 DOI [10.1021/jf981149l](https://doi.org/10.1021/jf981149l).
- Liong MT, Shah NP. 2005. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *Journal of Dairy Science* **88**(1):55–66 DOI [10.3168/jds.S0022-0302\(05\)72662-X](https://doi.org/10.3168/jds.S0022-0302(05)72662-X).
- Mejía-León ME, Barca AM. 2015. Diet, microbiota and immune system in type 1 diabetes development and evolution. *Nutrients* **7**(11):9171–9184 DOI [10.3390/nu7115461](https://doi.org/10.3390/nu7115461).
- Ménard S, Candalh C, Bambou JC, Terpend K, Cerf-Bensussan N, Heyman M. 2004. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. *Gut* **53**(6):821–828 DOI [10.1136/gut.2003.026252](https://doi.org/10.1136/gut.2003.026252).

- Moya-Pérez A, Neef A, Sanz Y. 2015. Bifidobacterium pseudocatenulatum CECT, 7765 reduces obesity-associated inflammation by restoring the lymphocyte-macrophage balance and gut microbiota structure in high-fat diet-fed mice. *PLOS ONE* 10(7):e0126976 DOI 10.1371/journal.pone.0126976.
- NCD Risk Factor Collaboration (NCD-RisC). 2016. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *The Lancet* 387(10027):1513–1530 DOI 10.1016/S0140-6736(16)00618-8.
- Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyötyläinen T, Nielsen T, Jensen BA, Forslund K, Hildebrand F, Prifti E, Falony G, Le Chatelier E, Levenez F, Dore J, Mattila I, Plichta DR, Poho P, Hellgren LI, Arumugam M, Sunagawa S, Vieira-Silva S, Jørgensen T, Holm JB, Trost K, MetaHIT Consortium, Kristiansen K, Brix S, Raes J, Wang J, Hansen T, Bork P, Brunak S, Oresic M, Ehrlich SD, Pedersen O. 2016. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 535(7612):376–381 DOI 10.1038/nature18646.
- Pimentel G, Burton KJ, Rosikiewicz M, Freiburghaus C, Von AU, Münger LH, Pralong FP, Vionnet N, Greub G, Badertscher R, Vergères G. 2017. Blood lactose after dairy product intake in healthy men. *British Journal of Nutrition* 118(12):1070–1077 DOI 10.1017/S0007114517003245.
- Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, Chilloux J, Ottman N, Duparc T, Lichtenstein L, Myridakis A, Delzenne N, Klievink J, Bhattacharjee A, van der Ark K, Aalvink S, Martinez L, Dumas M-E, Maiter D, Loumays A, Hermans MP, Thissen J-P, Belzer C, De Vos WM, Cani PD. 2017. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nature Medicine* 23(1):107–113 DOI 10.1038/nm.4236.
- Prattichizzo F, Giuliani A, Mensà E, Sabbatinelli J, De Nigris V, Rippo MR, La Sala L, Procopio AD, Olivieri F, Ceriello A. 2018. Pleiotropic effects of metformin: shaping the microbiome to manage type 2 diabetes and postpone ageing. *Ageing Research Reviews* 48:87–98 DOI 10.1016/j.arr.2018.10.003.
- Pussinen PJ, Havulinna AS, Lehto M, Sundvall J, Salomaa V. 2011. Endotoxemia is associated with an increased risk of incident diabetes. *Diabetes Care* 34(2):392–397 DOI 10.2337/dc10-1676.
- Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J. 2012. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490(7418):55–60 DOI 10.1038/nature11450.
- Rajkumar H, Mahmood N, Kumar M, Varikuti SR, Challa HR, Myakala SP. 2014. Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial. *Mediators of Inflammation* 2014(9):348959 DOI 10.1155/2014/348959.
- Resta-Lenert S, Barrett KE. 2003. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). *Gut* 52(7):988–997 DOI 10.1136/gut.52.7.988.
- Robbe-Masselot C, Capon C, Coddeville B, Michalski J-C. 2004. Structural diversity and specific distribution of O-glycans in normal human mucins along the intestinal tract. *Biochemical Journal* 384(2):307–316 DOI 10.1042/bj20040605.

- Sashihara T, Sueki N, Ikegami S. 2006. An analysis of the effectiveness of heat-killed lactic acid bacteria in alleviating allergic diseases. *Journal of Dairy Science* **89**(8):2846–2855 DOI [10.3168/jds.S0022-0302\(06\)72557-7](https://doi.org/10.3168/jds.S0022-0302(06)72557-7).
- Shang Q, Shan X, Cai C, Hao J, Li G, Yu G. 2016. Dietary fucoidan modulates the gut microbiota in mice by increasing the abundance of Lactobacillus and Ruminococcaceae. *Food & Function* **7**(7):3224–3232 DOI [10.1039/C6FO00309E](https://doi.org/10.1039/C6FO00309E).
- Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, Bae JW. 2014. An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* **63**(5):727–735 DOI [10.1136/gutjnl-2012-303839](https://doi.org/10.1136/gutjnl-2012-303839).
- Sonnenburg JL, Bäckhed F. 2016. Diet–microbiota interactions as moderators of human metabolism. *Nature* **535**(7610):56–64 DOI [10.1038/nature18846](https://doi.org/10.1038/nature18846).
- Tai N, Wong FS, Wen L. 2015. The role of gut microbiota in the development of type 1, type 2 diabetes mellitus and obesity. *Reviews in Endocrine and Metabolic Disorders* **16**(1):55–65 DOI [10.1007/s11154-015-9309-0](https://doi.org/10.1007/s11154-015-9309-0).
- Tazuke Y, Wasa M, Satoko N, Fukuzawa M. 2011. Protective mechanism of glutamine on the expression of proliferating cell nuclear antigen after cisplatin-induced intestinal mucosal injury. *Pediatric Surgery International* **27**(2):151–158 DOI [10.1007/s00383-010-2798-8](https://doi.org/10.1007/s00383-010-2798-8).
- Terpend K, Blaton M-A, Candalh C, Wal J-M, Pochart P, Heyman M. 1999. Intestinal barrier function and cow's milk sensitization in guinea pigs fed milk or fermented milk. *Journal of Pediatric Gastroenterology & Nutrition* **28**(2):191–198 DOI [10.1097/00005176-199902000-00019](https://doi.org/10.1097/00005176-199902000-00019).
- Tikellis C, Wookey PJ, Candido R, Andrikopoulos S, Thomas MC, Cooper ME. 2004. Improved islet morphology after blockade of the renin-angiotensin system in the ZDF rat. *Diabetes* **53**(4):989–997 DOI [10.2337/diabetes.53.4.989](https://doi.org/10.2337/diabetes.53.4.989).
- Tilg H, Moschen AR. 2014. Microbiota and diabetes: an evolving relationship. *Gut* **63**(9):1513–1521 DOI [10.1136/gutjnl-2014-306928](https://doi.org/10.1136/gutjnl-2014-306928).
- Ubeda C, Bucci V, Caballero S, Djukovic A, Toussaint NC, Equinda M, Lipuma L, Ling L, Gobourne A, No D, Taur Y, Jenq RR, Van Den Brink MR, Xavier JB, Pamer EG. 2013. Intestinal microbiota containing *Barnesiella* species cures vancomycin-resistant *Enterococcus faecium* colonization. *Infection and Immunity* **81**(3):965–973 DOI [10.1128/IAI.01197-12](https://doi.org/10.1128/IAI.01197-12).
- Vintiñi EO, Medina MS. 2011. Host immunity in the protective response to nasal immunization with a pneumococcal antigen associated to live and heat-killed *Lactobacillus casei*. *BMC Immunology* **12**(1):46 DOI [10.1186/1471-2172-12-46](https://doi.org/10.1186/1471-2172-12-46).
- Winer DA, Luck H, Tsai S, Winer S. 2016. The intestinal immune system in obesity and insulin resistance. *Cell Metabolism* **23**(3):413–426 DOI [10.1016/j.cmet.2016.01.003](https://doi.org/10.1016/j.cmet.2016.01.003).
- Yeung C-Y, Chan W-T, Jiang C-B, Cheng M-L, Liu C-Y, Chang S-W, Chiau J-SC, Lee H-C. 2015. Amelioration of chemotherapy-induced intestinal mucositis by orally administered probiotics in a mouse model. *PLOS ONE* **10**(9):e0138746 DOI [10.1371/journal.pone.0138746](https://doi.org/10.1371/journal.pone.0138746).
- Zhang L, Li N, Caicedo R, Neu J. 2005. Alive and dead *Lactobacillus rhamnosus* GG decrease tumor necrosis factor-alpha-induced interleukin-8 production in Caco-2 cells. *Journal of Nutrition* **135**(7):1752–1756 DOI [10.1093/jn/135.7.1752](https://doi.org/10.1093/jn/135.7.1752).
- Zhang Q, Sun X, Xiao X, Zheng J, Li M, Yu M, Ping F, Wang Z, Qi C, Wang T. 2016. Maternal chromium restriction leads to glucose metabolism imbalance in mice offspring through insulin signaling and Wnt signaling pathways. *International Journal of Molecular Sciences* **17**(10):1767 DOI [10.3390/ijms17101767](https://doi.org/10.3390/ijms17101767).
- Zorzela L, Ardestani SK, McFarland LV, Vohra S. 2017. Is there a role for modified probiotics as beneficial microbes: a systematic review of the literature. *Beneficial Microbes* **8**(5):739–754 DOI [10.3920/bm2017.0032](https://doi.org/10.3920/bm2017.0032).