Research Article

Preliminary Evaluation of Potential Properties of Three Probiotics and Their Combination with Prebiotics on GLP-1 Secretion and Type 2 Diabetes Alleviation

Ran Xiao,1,2 Ran Wang,1 Shusen Li,2 Xiaohong Kang,2 Yimei Ren,1 Erna Sun,2 Chenyuan Wang,2 Jingjing He,1 and Jing Zhan1

1Key Laboratory of Precision Nutrition and Food Quality, Department of Nutrition and Health, China Agricultural University, Beijing 100193, China
2Mengniu Hi-Tech Dairy Product Beijing Co., Ltd., Beijing 101100, China

Correspondence should be addressed to Jing Zhan; jingzhan@cau.edu.cn

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Type 2 diabetes (T2D) is a disease of global concern characterized by hyperglycemia and insulin resistance. Many studies found that glucagonlike peptide-1 (GLP-1) is an incretin hormone that can alleviate hyperglycemia and T2D. Recently, probiotics and their combination with prebiotics have been found to show great potentials of blood glucose regulation and T2D alleviation. Given the important role of GLP-1 in T2D, screening probiotics with the capacity of promoting GLP-1 secretion is of great help for providing a novel application of T2D treatment. In the current study, we evaluated the effects of three probiotics, namely, Lactobacillus paracasei LC-37 (LC-37), Bifidobacterium animals MN-Gup (MN-Gup), and Bifidobacterium longum BBMN68 (BBMN68), and their combination with prebiotics on promoting GLP-1 secretion using NCI-H716 cells. The results showed that LC-37 and MN-Gup could stimulate more GLP-1 secretion in NCI-H716 cells, but BBMN68 had no significant effect. Further evaluation suggested that the two combinations of LC-37 with isomaltooligosaccharide (IMO) and MN-Gup with galactooligosaccharide (GOS) had the best performance on promoting GLP-1 secretion in vitro. Subsequently, the effects of the two combinations on promoting GLP-1 secretion and alleviating T2D were investigated in vivo using high fat diet (HFD) and streptozotocin (STZ) treated rats. The results showed that the two combinations could significantly reduce fasting blood glucose levels, improve insulin resistance, and modulate serum lipid profiles in HFD/STZ-treated rats. These results will help understand the potential of promoting GLP-1 secretion of LC-37 and MN-Gup and provide theoretical basis for their applications in fermented milk or other foods.

1. Introduction

Diabetes is a serious metabolic disorder disease characterized by chronic hyperglycemia because of insufficient insulin production (Type 1) or insulin resistance (Type 2) [1, 2]. Type 2 diabetes (T2D) has become one of the most prevalent diseases worldwide and can lead to serious complications, such as cardiovascular disorders, renal failure, and blindness [3]. The prevention and treatment of T2D have drawn public attentions [4]. Probiotics, particularly Lactobacilli and Bifidobacteria, have recently emerged as the potential adjunctive therapy with proven efficacy demonstrated in various in vitro and in vivo models for alleviating the development of diabetes [5, 6]. Lactobacillus spp., including L. rhamnosus, L. paracasei, L. plantarum, and L. casei, could effectively reduce blood glucose concentrations in T2D animal models [7–11]. Bifidobacteria were also demonstrated to normalize the insulin sensitivity and fasting hyperinsulinemia and improve the tissue inflammation by reducing the expression of major proinflammatory cytokines [12–14].

Gut can secret incretin hormones and thus impact the occurrence of T2D in mammals [15, 16]. One of the most important incretin hormones is glucagonlike peptide-1...
Lactobacillus paracasei understand the potentials of promoting GLP-1 secretion of NCI-H716 cells. Probiotics and their combinations with prebiotics using their property on promoting GLP-1 secretion of these three recent theories consider probiotics benefiting functional in vivo (HFD) and streptozotocin (STZ). However, their potential of alleviating T2D was largely considered as probiotics benefiting functional with matrigel at a density of 1.5 × 10⁶ cells per well and cultured 48 h before the assay. Then, supernatants were replaced by Krebs-Ringer buffer (KRB) under pH 7.4 (128.8 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L KH₂PO₄, 1.2 mmol/L MgSO₄, 2.5 mmol/L CaCl₂, 5 mmol/L NaHCO₃, and 10 mmol/L HEPES) with or without probiotics. Different probiotics suspended in KRB were designed at low (5 × 10⁵ CFU/mL) and high (5 × 10¹⁰ CFU/mL) doses. Control group was the supernatant of NCI-H716 cells incubated with KRB. After incubating for 2 h, the supernatant was collected in 1.5 mL microcentrifuge tube containing 50 µg/mL phenylmethylsulfonyl fluoride (PMSF) and 10 µg/mL dipeptidyl peptidase-4 (DPP-4) inhibitor (Sigma-Aldrich, St. Louis, MO, USA) and was centrifuged at 1000 × g at 4°C for 10 min to remove cells and lactobacilli debris. The supernatant was measured for GLP-1 concentrations using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Merck, Billerica, MA, USA).

2.2. NCI-H716 Cell Culture. NCI-H716 cells were cultured in RPMI 1640 (Gibco, Life Technologies, Ghent, Belgium) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% penicillin/streptomycin (v/v) at 37°C, 5% CO₂ until 80%–85% confluent.

2.3. GLP-1 Secretion Assay in NCI-H716 Cells. The GLP-1 secretion assay was based on Reimer’s method with modification [32]. The NCI-H716 cells were seeded in 12-well plates coated with matrigel at a density of 1.5 × 10⁶ cells per well and cultured 48 h before the assay. Then, supernatants were replaced by Krebs-Ringer buffer (KRB) under pH 7.4 (128.8 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L KH₂PO₄, 1.2 mmol/L MgSO₄, 2.5 mmol/L CaCl₂, 5 mmol/L NaHCO₃, and 10 mmol/L HEPES) with or without probiotics. Different probiotics suspended in KRB were designed at low (5 × 10⁵ CFU/mL) and high (5 × 10¹⁰ CFU/mL) doses. Control group was the supernatant of NCI-H716 cells incubated with KRB. After incubating for 2 h, the supernatant was collected in 1.5 mL microcentrifuge tube containing 50 µg/mL phenylmethylsulfonyl fluoride (PMSF) and 10 µg/mL dipeptidyl peptidase-4 (DPP-4) inhibitor (Sigma-Aldrich, St. Louis, MO, USA) and was centrifuged at 1000 × g at 4°C for 10 min to remove cells and lactobacilli debris. The supernatant was measured for GLP-1 concentrations using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Merck, Billerica, MA, USA).

2.4. Proliferation of Prebiotics on Probiotics. Apple fiber, xylitol, and galactooligosaccharide (GOS) were selected to investigate their effects on proliferation of probiotics. Prebiotics were sterilized before use, and their concentration was 1.5% for initial screening. Then, 1% of the activated bacteria obtained were placed in the MRS basic medium containing glucose or prebiotics as carbon sources, or in a carbon source-free medium, and statically cultivated at 37°C. The absorbance of the fermentation broth was measured at 660 nm at 0 h, 4 h, 8 h, 12 h, and 24 h, respectively, and the prebiotic index (PI) was calculated according to the following formula [33]:

\[
PI = \frac{(A_{PP24} - A_{PP0}) - (A_{PN24} - A_{PN0})}{(A_{PG24} - A_{PG0}) - (A_{PN24} - A_{PN0})}. 
\]

2.5. Animal Housing and Breeding. Male Sprague Dawley (SD) rats with body weight ranging from 170–190 g (8 weeks old) were purchased from Vital River Laboratory Animal Co. Ltd. (Beijing, China) and kept in the animal room with...
constant temperature (22 ± 2°C) and humidity (55 ± 5%) in 12 h light-dark cycle. All animals were fed with food and water *ad libitum*. Body weight of rats was measured twice a week. The study was approved by the institutional animal ethics committee (Approval Number: PONY-2020-FL-52).

2.6. *High Fat Diet (HFD) and Streptozotocin- (STZ-) Treated Rats for T2D Model.* T2D model and treatment design has been presented in Figure 1. Following 1-week acclimatization period, rats were randomly divided into five groups (*n = 5* for each group): the control group was fed with normal chow (NC); and the other 4 groups were fed with high fat diet (HFD) for 5 weeks, followed by intraperitoneally injection with 30 mg/kg b.w. streptozotocin (STZ) to develop diabetic model [34]. During 7-week treatment period, the model group received a vehicle gavage with sterilized saline, and the other HFD/STZ-treated rats were treated with combination of LC-37 and IMO, or combination of MN-Gup and GOS. The rats with injection of 0.18 mg/kg b.w. GLP-1 were used as positive control (PC) group. After 12 h fasting, blood glucose of rats was detected, and then they were euthanized for collecting blood at the 14th week.

2.7. *Oral Glucose Tolerance Test (OGTT).* To investigate the effects of HFD and STZ administrations on T2D development, oral glucose tolerance test (OGTT) was conducted after 1-week STZ treatment. Rats were deprived of food for 12 h, and OGTT was performed after gavage with 2 g/kg b.w. glucose, and their blood glucose levels were measured via tail vein sampling at 30, 60, 90, and 120 min. The area under curve (AUC) of glucose concentration-time was calculated to characterize oral glucose tolerance. At the end of treatment (the 14th week), OGTT of all groups were conducted by the same method.

2.8. *Blood Parameters.* Blood samples were subjected to analysis of the serum levels of cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Insulin in rat serum was detected, and homeostasis model assessment of insulin resistance (HOMA-IR) was measured with the following formula:

\[
\text{HOMA-IR} = \frac{\text{Fasting blood glucose} \times \text{Fasting insulin}}{22.5}
\]

The units of fasting blood glucose and fasting insulin were mmol/L and mU/L, respectively.

2.9. *Statistical Analysis.* Differences between treatments were determined using the one-way ANOVA with Duncan analysis. Statistical significance was defined as *p < 0.05*. All statistical analyses were performed using Origin 2019 software (OriginLab Corporation, Massachusetts, USA).

3. Results

3.1. *Effects of Probiotic Strains on Intestinal GLP-1 Secretion In Vitro.* NCI-H716 cell line is a common model available for the *in vitro* study of GLP-1 regulation [35]. The three probiotic strains, namely, LC-37, MN-Gup, and BBMN68, were, respectively, incubated in NCI-H716 cells at low

![Figure 1: Experimental design.](image-url)

![Figure 2: Effects of three probiotic strains on GLP-1 secretion in NCI-H716 cells at low (5 × 10^9 CFU/mL) and high (5 × 10^10 CFU/mL) doses. Control group is incubated with PBS. Data are shown as means ± standard deviations (SD), and different lowercase letters indicate significant differences, *p < 0.05* (*n = 3*).](image-url)
results indicated that LC-37 and MN-Gup could significantly stimulate GLP-1 secretion in NCI-H716 cells at both low and high doses, and the high doses had more significant performance than the low doses (Figure 2). In contrast, BBMN68 did not significantly promote GLP-1 secretion compared to the control. Therefore, MN-Gup and LC-37 strains were used for the following evaluation.

3.2. Selection of Prebiotics with the Abilities to Promote Probiotics Growth In Vitro. The prebiotic index (PI) could be used to evaluate the relative growth-promoting capability of a prebiotic to that of glucose [33]. Four common prebiotics, whose added contents were 1.5%, were, respectively, used as carbon source for LC-37 and MN-Gup, and their prebiotic effects were investigated by evaluating PI. As shown in Figure 3(a), XOS, IMO, and GOS could promote the growth of LC-37 and MN-Gup, while apple fiber had the opposite effects. To screen an optimal concentration of prebiotics, the prebiotic effects of 0.75%, 1.5%, and 3% of XOS, IMO, and GOS were investigated. The results showed that XOS and GOS at 1.5% had the best prebiotic effects on LC-37, and the overall performance of GOS was better than that of XOS (Figure 3(b)). IMO at 3% had the best prebiotic effect, but 1.5% IMO also had a prebiotic effect (Figure 3(b)), so the optimum concentration could be chosen at 1.5%. For MN-Gup, the optimal concentration was 1.5%, and the overall prebiotic effect was GOS > IMO > XOS (Figure 3(c)). Thus, IMO and GOS at 1.5% were selected for the following evaluation.

3.3. Effects of LC-37 and MN-Gup with Prebiotics on Intestinal GLP-1 Secretion In Vitro. To investigate whether the supplement of IMO and GOS could help LC-37 and MN-Gup promote intestinal GLP-1 secretion, GLP-1 secretion of NCI-H716 cells incubated with LC-37 and MN-Gup combined with 1.5% IMO or GOS was assessed. As shown in Figure 4, LC-37 with IMO could significantly increase GLP-1 secretion, but LC-37 with GOS did not induce significant promotion on GLP-1 secretion when compared to LC-37 alone. MN-Gup with IMO or GOS could significantly promote GLP-1 secretion when compared to MN-Gup alone, but GOS had a better performance than IMO. Many reports have verified the important role of GLP-1 in glucose homeostasis and insulin sensitivity [36, 37], inferring that the combination of LC-37 and IMO or combination of MN-
GLP-1 secretion (fold of control)

![Figure 4: Effects of LC-37 and MN-Gup with prebiotics on intestinal GLP-1 secretion in NCI-H716 cells. The added contents of IMO and GOS were 1.5%. Control group is incubated with PBS. Data are shown as means± standard deviations (SD), and different lowercase letters indicate significant differences, p<0.05 (n=3).](image)

Gup and GOS may regulate glucose homeostasis and insulin resistance by promoting GLP-1 secretion, thereby alleviating T2D.

### 3.4. Effects of LC-37 and MN-Gup with Prebiotics on the Symptoms of T2D in HFD/STZ-Treated Rats

A T2D model using HFD/STZ-treated rats was established to investigate whether the combination of LC-37 and IMO (LC-37 ± IMO) or combination of MN-Gup and GOS (MN-Gup + GOS) could promote GLP-1 secretion and alleviate T2D *in vivo*. Treatment of GLP-1 was used as positive control. As shown in Figure 5(a), the initial body weights were similar for all groups. After treatment of STZ, body weights of rats were significantly reduced compared to the normal rats (NC), which was consistent with the sudden weight loss of T2D symptoms. Moreover, glucose tolerance of rats was significantly reduced compared to the NC group, and the index of HOMA-IR was significantly reduced by treatment with LC-37 ± IMO and MN-Gup + GOS (Figure 5(e)). These results indicated that LC-37 ± IMO and MN-Gup + GOS could reduce the hyperglycemia and improve insulin resistance.

Moreover, the occurrence of T2D is often accompanied by dyslipidemia such as the accumulation of TG and TC. As shown in Figure 5(f), the model group had significantly higher TG, TC, and LDL-C and lower HDL-C, suggesting that dyslipidemia was induced in these rats. Treatment with LC-37 ± IMO and MN-Gup + GOS could significantly improve dyslipidemia by reducing TG, TC, and LDL-C and enhancing HDL-C.

### 3.5. Effects of LC-37 and MN-Gup with Prebiotics on Serum GLP-1 in HFD/STZ-Treated Rats

As shown in Figure 6, serum GLP-1 concentration was significantly reduced by HFD and STZ treatment. Treatment with LC-37 ± IMO and MN-Gup + GOS could significantly enhance the GLP-1 concentration in serum, which were consistent with their properties on promoting GLP-1 secretion *in vitro*. It was inferred that treatment with LC-37 ± IMO and MN-Gup + GOS had a potential of alleviating T2D via promoting GLP-1 level.

### 4. Discussion

It is found that GLP-1 secretion is decreased in T2D, thus making it a logical target for novel treatments of T2D [38]. Screening probiotics with capacity of promoting GLP-1 secretion is of great significance for providing a novel application on T2D treatment. *Bifidobacterium* and *Lactobacillus* have been demonstrated to be two classes of probiotics playing potential clinical roles [39]. For instance, *Bifidobacterium longum* was found to maintain gut barrier and regulate immunity by producing exopolysaccharides (EPS) in intestinal pathogen infection and even show potentials of assisting in decreasing coronavirus disease 2019 (COVID-19) infection [39, 40]. *Lactobacillus paracasei* LC-37, *Bifidobacterium* animals MN-Gup, and *Bifidobacterium longum* BBMN68 have been recently demonstrated as rising stars of probiotics and showed wide application potential in fermented milk and other foods due to their regulations on functional dyspepsia, energy metabolism, and immunity [29–31]. Herein, we evaluated these strains according to their ability to stimulate the production of GLP-1 based on the *in vitro* model using NCI-H716 cells, used PI values to discover strain-specific prebiotics and their concentration, and eventually verified the symptoms-reduced effect of probiotics and prebiotics with T2D rats induced by STZ. Our results showed that LC-37 and MN-Gup but not BBMN68 displayed good performance on promoting GLP-1 secretion, and the two combinations of LC-37 ± IMO and MN-Gup + GOS could normalize hyperglycemia and possibly prevent diabetes complications in diabetic rats.
NCI-H716 cell line is commonly used to study GLP-1 secretion due to their high GLP-1 secretory capacity [35]. LC-37 and MN-Gup showed their strong ability to simulate the production of GLP-1 in NCI-H716 cells (Figure 2), which could be further elevated by prebiotics (Figure 4). Prebiotics can be fermented by gut microbiota and produce

![Graph showing body weight changes over weeks](a)

![Graph showing AUC glu (mmol/L.min)](b)

![Graph showing fasting blood glucose level (mmol/L)](c)

![Graph showing insulin level (mU/L)](d)

![Graph showing HOMA-IR](e)

![Graph showing serum lipid profiles (mmol/L)](f)

**Figure 5:** Effects of LC-37 and MN-Gup with prebiotics on (a) body weight, (b) glucose tolerance assessed by oral glucose tolerance test (OGTT), (c) fasting blood glucose level, (d) insulin level, (e) HOMA-IR, and (f) serum lipid profiles in HFD/STZ-treated rats. Data are shown as means ± standard deviations (SD), and different lowercase letters indicate significant differences, $p < 0.05$ ($n = 5$).
SCFAs, mainly including acetic acid, propionic acid, and butyric acid [41]. SCFAs can activate GLP-1 secretion, which stimulates the elevation of insulin production and increase insulin sensitivity by acting on specific G protein-coupled receptors, GPCR 43 and GPCR 41 on enteroendocrine L-cells [42, 43]. IMO and GOS have been reported to be effective at increasing numbers of probiotics and promoting SCFAs generation [44]. LC-37 + IMO and MN-Gup + GOS might promote GLP-1 secretion via triggering SCFAs generation.

Furthermore, an in vivo study with HFD/STZ-induced T2D rats was conducted, and the results revealed that administering LC-37 + IMO and MN-Gup + GOS could alleviate T2D, including lowering the level of fasting blood glucose and insulin resistant, whose performance was similar to the positive control of GLP-1 treatment (Figure 5). Pancreatic β-cell dysfunction and insulin resistance are vital mechanisms in the development of diabetes [45]. Surprisingly, the treatment with GLP-1 did not exhibit the significant effects on insulin level in serum (Figure 5(d)). STZ can cause pancreatic islet β-cell destruction and insulin secretion depletion, and its effect combining with long-term HFD may induce a state of the terminal stage of T2D and prevent the treatment with GLP-1 from reversing insulin secretion, which may be the reason why the treatment with GLP-1 could not promote a significant increase in insulin level [46]. Neither LC-37 + IMO nor MN-Gup + GOS significantly elevated insulin levels. These results may reveal that promoting insulin secretion was not the mechanism by which GLP-1, LC-37 + IMO, and MN-Gup + GOS alleviated T2D. HOMA-IR, which is related to fasting blood glucose and insulin levels, is commonly used as a procedure for estimating insulin resistance [47]. The reduction of fasting blood glucose and the normalization of HOMA-IR level both proved that administration of LC-37 and MN-Gup with prebiotics could improve insulin resistance (Figures 5(c)–5(e)). Blood lipids may directly interact with glycometabolism, and dyslipidemia is one of the complications of diabetes [48]. Dyslipidemia had also been observed in the HFD/STZ-treated rats, but LC-37 + IMO and MN-Gup + GOS administration caused significant reduction of the TC, TG, and LDL-C level and enhancement of HDL-C level (Figure 5(f)), supporting their effects on T2D alleviation. GLP-1 in serum of rats was significantly increased by LC-37 + IMO and MN-Gup + GOS treatment (Figure 6), which was consistent with the phenotype in NCI-H716 cells. LC-37 and MN-Gup with prebiotics may alleviate T2D by stimulating GLP-1 secretion.

There were some limitations in the current study. Firstly, how LC-37 and MN-Gup promote GLP-1 production is unclear. Secondly, the underlying mechanism of promoting GLP-1 secretion by LC-37 and MN-Gup with prebiotics was not explored in vivo. LC-37 and MN-Gup with prebiotics may promote GLP-1 production and alleviate T2D via regulating gut microbiota and SCFAs in rats, which need to be studied in the future.

Overall, the effects of Lactobacillus paracasei LC-37, Bifidobacterium animals MN-Gup, and Bifidobacterium longum BBMN68 and their combination with prebiotics on promoting GLP-1 secretion and alleviating T2D were evaluated using NCI-H716 cells and HFD/STZ-treated rats. The results showed that the two combinations of LC-37 with IMO and MN-Gup with GOS had the best performance on promoting GLP-1 secretion and alleviating T2D-related symptoms in both in vitro and in vivo models. These results are helpful in developing the application of LC-37 and MN-Gup on T2D treatment via targeting on promoting GLP-1 secretion.

Data Availability

The data used to support the findings of this study are included within the article.

Additional Points

(i) LC-37 and MN-Gup but not BBMN68 promoted GLP-1 secretion in NCI-H716 cells. (ii) LC-37 + IMO and MN-Gup + GOS significantly promoted GLP-1 secretion in NCI-H716 cells. (iii) LC-37 + IMO and MN-Gup + GOS raised GLP-1 level and alleviated T2D in T2D model rats.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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