Antidiabetic effect of *Lactobacillus casei* CCFM0412 in high-fat-fed, streptozotocin-induced type 2 diabetic mice

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1. Running head
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Abstract

Objective: To evaluate the antidiabetic effects of *Lactobacillus casei* CCFM0412 on mice with type 2 diabetes induced by a high-fat diet and streptozotocin.

Methods: Thirty two male C57BL/6J mice were assigned to four groups in this study. Type 2 diabetes was induced by feeding a high-fat diet and injection of streptozotocin. *L. casei* CCFM0412 was administered to mice at a dose of $10^9$ cfu/d·mouse for 12 weeks. Body weight, fasting and postprandial 2-h blood glucose, oral glucose tolerance, glycosylated hemoglobin (HbA1c), insulin, and glycogen in liver were measured. Endotoxin, tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) levels were determined. Lipid metabolic parameters including triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were also measured. The activities of glutathione peroxides (GSH-px), reactive oxygen species (ROS) and superoxide dismutase (SOD), and the levels of glutathione (GSH) and malondialdehyde (MDA) in liver were also determined. Pancreas injury was evaluated by histological analysis.

Results: At 13 weeks, *L. casei* CCFM0412 significantly decreased fasting and postprandial 2-h blood glucose, HbA1c, endotoxin, TNF-α, TG, TC, LDL-C, ROS, and MDA levels compared with the control group ($P < 0.05$). The values for insulin, IL-10, HDL-C, GSH-px, SOD, GSH, and glycogen were significantly increased at 13 weeks ($P < 0.05$). Islets of Langerhans in the *L. casei* CCFM0412 group were substantially protected from destruction compared with those in the control group.

Conclusion: *L. casei* CCFM0412 significantly improved glucose intolerance, dyslipidemia, immune-regulatory properties, and oxidative stress in high-fat-fed, streptozotocin-induced type 2 diabetic mice. The results provide a sound rationale for future clinical trials of oral administration of *L. casei* CCFM0412 for the primary
prevention of type 2 diabetes.

**Key words:** *Lactobacillus casei* CCFM0412; antidiabetic effect; pioglitazone; type 2 diabetes; blood glucose

**Introduction**

The incidence of type 2 diabetes mellitus, which is characterized by abnormally high blood glucose due to a relative deficiency of insulin [1], has rapidly increased in the world during the past few decades. It has been suggested that insulin resistance precedes the development of overt hyperglycemia, although the molecular basis for this has not been identified. In the liver, insulin resistance is translated into impaired suppression of glucose production due to hyperglycemia and glucose intolerance [2, 3]. The action of innate immune system is one of the biological mechanisms of insulin resistance. Many studies have shown that as markers of inflammation, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) are associated with type 2 diabetes and dyslipidemia [4, 5]. Interleukin-10 (IL-10), an anti-inflammatory cytokine that plays a key role in the regulation of the innate immune system, exerts powerful deactivating properties on the inflammatory host and potently inhibits the production of pro-inflammatory cytokines such as TNF-α [6].

There is also much experimental proof that oxidative stress plays an important role in the pathogenesis and progression of diabetes and its complications [7, 8].

Five classes of oral antidiabetic agents are available: α-glucosidase inhibitors, sulfonylurea, meglitinides, biguanides, and thiazolidinediones [9]. However, the use of these medicines has serious side effects and causes secondary failure, including bloating, flatulence and diarrhea [10]. Lactic acid bacteria (LAB) have none of these side effects, thus can be considered. In recent years, many studies have demonstrated that LAB are useful in preventing or decreasing the progression of diabetes. Yun et al.
found that the blood glucose levels in type 2 diabetic mice administered 
*Lactobacillus gasseri* BNR17 were lower than those in the control group. Tabuchi et 
al. [12] demonstrated that *Lactobacillus rhamnosus* GG cells significantly lowered 
blood glucose levels and improved hyperglycemia in neonatal streptozotocin-induced 
diabetic rats compared with the control group. Previous studies have shown that LAB 
can significantly decrease the oxidative stress of high fructose-induced diabetic rats 
and enhance pancreatic glutathione biosynthesis, thus reducing oxidative stress in the 
pancreas [13, 14]. LAB prevented or delayed the onset of diabetes in various 
experimental models in those studies. We hypothesized that LAB have antidiabetic 
ability by their α-glucosidase inhibitory activity in vivo, improving the plasma lipids, 
and increasing the ability of antioxidant in mice.

*L. casei* CCFM0412 was isolated from yogurt in our lab. Our previous 
experiments showed that this strain exhibited good probiotic properties including acid 
and bile salt tolerance, and antioxidant ability. Moreover, its cell-free supernatant 
inhibition rate of rat intestinal α-glucosidase was 30 %.( Journal of Chinese Institute 
of Food Science and Technology, accepted). Several studies have demonstrated that 
the association of oxidative stress markers with diabetes and ROS plays an important 
role in the regulation of insulin resistance [15-17]. Meanwhile, dyslipidemia is a 
common and important character in type 2 diabetes [18], Yadav et al. found that 
probiotic can improve dyslipidemia and delay the progression of high 
fructose-induced glucose intolerance in rats [13]. Our vitro study has demonstrated 
that *L. casei* 2W has an excellent ability to antioxidant. So in this study, we 
investigated whether *L. casei* CCFM0412 affected blood glucose levels and 
ameliorated the oral glucose tolerance in high-fat-fed, streptozotocin-induced type 2 
diabetic mice by improving dyslipidemia, immunoregulatory properties, and oxidative
stress.

Materials and methods

Preparation of bacteria suspensions

*L. casei* CCFM0412 samples for administration to animals were prepared by suspending lyophilized bacteria powered with skimmed milk as its protectant [11]. Colony counting was conducted before the animal experiments to ensure the numbers of surviving bacteria were adjusted to $4 \times 10^9$ cfu/mL.

Experimental animals

Thirty two, 3-weeks-old male C57BL/6J mice were obtained from the Shanghai Laboratory Animal Center (Shanghai, China). The treatment protocol listed in Figure 1. The mice were housed in an animal room at constant temperature (22 ± 2°C) and humidity (55 ± 5%) under a 12-h light/12-h dark cycle in the animal facility at the Jiangnan University. The mice were randomly divided into 4 groups (n = 8 per group) as follows: normal (N) group, normal mice without diabetes; control (C) group, mice with diabetes but no treatment; pioglitazone (P) group, mice with diabetes and treatment with pioglitazone (one drug for treating diabetes); *L. casei* CCFM0412 (L) group, mice with diabetes and treatment with *L. casei* CCFM0412. A diet of normal chow (Table 1A) and water were freely accessible to the mice for 1 week (not shown in Figure 1). Then, the C, P, and L groups were fed a high-fat diet (Table 1B), and the N group continued to consume normal chow for 12 weeks. The L group mice were administered $4 \times 10^9$ cfu/mL *L. casei* CCFM0412 250 µL per day for 12 weeks. At 3 weeks, the C, P, and L group mice were intraperitoneally injected with streptozotocin (Sigma, St. Louis, MO, USA) freshly dissolved in 50 mmol/L citrate buffer (pH 4.5)
at a dose of 100 mg/kg body weight, whereas the N group mice received 0.9% saline alone [19]. After 1 week (week 4), pioglitazone was administered to the P group mice at a dose of 10 mg/kg body weight. Body weights were measured once a week. Fasting and postprandial 2-h blood glucose levels were analyzed with a glucometer (HMD Biomedical, Taiwan, China) once a week from blood collected from the tail vein of the mice. This study was approved by the animal Ethics Committee of Jiangnan University, China (JN No. 26 2012).

Oral glucose tolerance test

The oral glucose tolerance tests (OGTT) is primary outcome which has often been used to derive estimates of the relative roles of insulin secretion and insulin resistance in population studies of glucose tolerance [20, 21]. OGTT were performed in weeks 4 and 13. Before each test, mice were fasted for 12 h and blood glucose was measured (time = 0). The glucose solution was prepared at a concentration of 2 g/kg body weight and administered to the mice. Then, blood glucose values were analyzed at 15, 30, 60, 90, and 120 min [13].

Blood and tissue sample collection

On the last day of the experiment (week 13), blood samples were collected from the orbital venous plexus of 12-h fasted and anesthetized animals. The blood samples were centrifuged at 4000 × g for 10 min at 4°C, and serum was obtained to analyze levels of glycosylated hemoglobin (HbA1c), insulin, endotoxin, TNF-α, IL-10, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Then, the mice were sacrificed by cervical dislocation. The liver and pancreatic tissues were immediately removed and rinsed with cold 0.9% saline. The liver was then chopped into pieces
before being stored at -80°C until use, and the pancreas was fixed in 10% formalin-saline [13].

Determination of HbA1c, insulin, endotoxin, TNF-α, and IL-10

The values of HbA1c reflect overall glycemic exposure over the past 2-3 months and are determined by both fasting and postprandial 2-h blood glucose levels [22]. Insulin is produced by the pancreas and that regulates the level of glucose in the blood. TNF-α and endotoxin are two contributing factors in insulin resistance and dyslipidemia [23]. IL-10 plays an important role in the regulation of the innate immune system to prevent the development of diabetes in nonobese diabetic mice [24]. The levels of HbA1c, insulin, TNF-α, and IL-10 were measured according to the recommendations of the manufacturer using enzymatic kits purchased from Abcam (Cambridge, UK), whereas the value of endotoxin was determined by an ELISA kit which purchased from Usen Life Science (Wuhan, China).

Determination of plasma lipids

Abnormal plasma lipids in diabetic patients include elevated LDL-C and TG, and reduced levels of HDL-C. These are associated with the onset of diabetes [25]. The serum levels of TG, TC, LDL-C, and HDL-C were determined by enzymatic kits produced by Boster Bio-engineering Company Ltd. (Wuhan, China).

Determination of GSH-px, ROS, SOD, GSH, MDA, and glycogen

Accumulating evidences indicated that the generation of oxidative stress may play an important role in the etiology of diabetic complications [26]. Glycogen is mainly stored in the liver and the muscles, which is useful for providing a readily available source of glucose for the body. The activities of glutathione peroxides
(GSH-px), reactive oxygen species (ROS), superoxide dismutase (SOD), and the 
levels of glutathione (GSH), malondialdehyde (MDA), and glycogen in the livers 
were measured according to the recommendations of the manufacturer, using 
enzymatic kits produced by Boster Bio-engineering Company Ltd.

**Histopathological examination**

The pancreases were fixed for 48 h in 10% formalin-saline, embedded in paraffin, 
serially cut at 5-µm thickness with a rotary microtome, and routinely stained with 
hematoxylin-eosin (H-E) for light microscopy examination (DM2000; Leica, 
Bensheim, Germany).

**Statistical analysis**

A statistical analysis of the obtained data was carried out using SPSS 16.0 for 
Windows (SPSS Inc., Chicago, IL). The weight and glucose along time between 
groups were analyzed through two-way ANOVA for repeated measurements. 
Differences between the means of the tests were evaluated by Tukey’s test. 
Correlations between parameters where assessed by Pearson’s correlation test. A value 
of $P < 0.05$ was regarded as statistically significant.

**Results**

Figure 2 presents change in body weight. The two-way analysis of variances for 
repeated measurements revealed a significant overall effect of time ($F = 99.28$; 
$P < 0.001$) and group ($F = 50.40$; $P < 0.001$). The body weights of the mice in all four 
groups gradually increased over the period of the experiment, with that in the C group 
increasing slowly after 7 weeks. After 3 weeks, body weights in the N group were 
higher than C group. However, at 13 weeks, body weights in the L and C groups were
lower by 4% and 3%, respectively, versus those at 12 weeks. This weight loss did not occur in the N group. In addition, the body weights in the L group significantly higher \((P < 0.05)\) than those in the C group at 3-5 weeks and 9 weeks.

Figure 3A presents changes in the levels of fasting blood glucose. The two-way analysis of variances for repeated measurements revealed a significant overall effect of time \((F = 139.68; P<0.001)\) and group \((F= 1867; P<0.001)\). Fasting blood glucose levels of the N and L groups were significantly lower \((P < 0.05)\) than that of the C group after 4 weeks, whereas they were significantly lower \((P < 0.05)\) after 5 weeks in the P group. The fasting blood glucose level of the C group peaked \((19.9 \text{ mmol/L})\) at 8 weeks. In contrast, the values in the L group were maintained at a relatively low level.

Figure 3B presents changes in the levels of postprandial 2-h blood glucose. The two-way analysis of variances for repeated measurements revealed a significant overall effect of time \((F = 259.73; P<0.001)\) and group \((F= 2786; P<0.001)\). After 4 weeks, postprandial 2-h blood glucose levels of the N and L groups were significantly lower \((P < 0.05)\) than those of the C group, and they were significantly lower \((P < 0.05)\) after 5 weeks in the P group. At 9 weeks, the postprandial 2-h blood glucose levels in C group peaked \((29.6 \text{ mmol/L})\).

Figure 4 shows the results of the OGTT at week 4 (1 week after streptozotocin injection) and at the end of the experiment (week 13). Glucose tolerances were impaired at 4 weeks in the C, P, and L groups. The AUC_{glucose} value in the C group was significantly higher than that in the N group \((P < 0.05)\). The AUC_{glucose} value in the L group was significantly lower \((18\%)\) than that in the C group \((P < 0.05)\) (Figure 4A). At 13 weeks, the glucose tolerance of the L and P groups improved compared with that in the C group. The AUC_{glucose} of the L group \((30\%)\) was also significantly lower than that of the C group \((P < 0.05)\) (Figure 4B).
Table 2 shows that the HbA1c levels in the C group were significantly higher (53%) than those in the N group ($P < 0.05$), whereas the HbA1c levels in the L group were clearly lower (23%) than those in the C group. There were no significant differences ($P > 0.05$) in HbA1c levels between the P and L groups. After 12 weeks of *L. casei* CCFM0412 administration, serum insulin and hepatic glycogen levels increased significantly (to 45% and 83%, respectively) in the L group compared with the levels in the C group ($P < 0.05$). No significant differences were observed in serum insulin and liver glycogen levels between the N and P groups ($P > 0.05$).

Table 2 presents the values of the lipid metabolic parameters after 12 weeks of *L. casei* CCFM0412 administration. There were no significant differences ($P > 0.05$) in the levels of TG between the C and N groups, whereas the values in the P and L groups were significantly lower (18% and 19%, respectively) compared with that in the C group ($P < 0.05$). The TC and LDL-C levels in the C group were significantly higher (15% and 23%, respectively) than those in the N group ($P < 0.05$), but no significant differences were found in the levels of LDL-C between the P and L groups ($P > 0.05$). Otherwise, the value of HDL-C in the C group was significantly lower (14%) than that in the N group ($P < 0.05$), and no significant differences were observed between the P and C groups ($P > 0.05$). The level of HDL-C in the L group was 19% higher than that in the C group.

As shown in Table 2, the activity of GSH-px and the level of GSH were significantly lower (28% and 15%, respectively) in the C group than in the N group ($P < 0.05$). In addition, the activities of GSH-px and the levels of GSH in the P and L groups were not significantly different when compared with those of the N group ($P > 0.05$). The activities of SOD in the C and P groups were significantly lower (35% and 19%, respectively) than those in the N group ($P < 0.05$), whereas no significant
differences were found between the L and N groups ($P > 0.05$). However, MDA levels were significantly higher in the C and P groups (34% and 19%, respectively) than that in the N group ($P < 0.05$), but no significant differences were found between the L and N groups ($P > 0.05$).

Figure 5 shows the values of endotoxin, TNF-α and IL-10 in mice administered *Lactobacillus casei* CCFM0412 for 13 weeks. We observed that the values of endotoxin and TNF-α in the C group were significantly higher (70% and 36%, respectively) than those in the N group ($P < 0.05$). However, these variables significantly decreased (both 21%) in the L group compared with those in the C group ($P < 0.05$, Figure 5A, B). The level of IL-10 in the C group was 17% lower than that in the N group, and no significant differences ($P > 0.05$) were observed in IL-10 between the N, P, and L groups (Figure 5C).

Histological analysis of the pancreases of the four groups showed substantial differences in the number and pattern of islets of Langerhans (Figure 6). A marked decrease (57.27%) in the number of islets of Langerhans was noted in the C group compared with that in the N group, and the C group also showed atrophy of the islets. The islet cell clusters were round or oval, dispersed in pancreatic acinar, and had clear boundaries in the N group compared with those in the C group (Figure 6a, b). In the P and L groups, the number of islet cells was 96±27 and 155±22 respectively, which were higher than those in C group.

**Discussion**

Recent studies have concentrated on LAB that can prevent or delay the onset of type 2 diabetes [8,13, 27]. In the present study, we found an antidiabetic effect of *L. casei* CCFM0412 on C57BL/6J mice with high-fat-fed, streptozotocin-induced type 2
diabetes. The body weights were significantly ($P < 0.001$) affected by time and groups.

Under normal circumstances, the body weight increased along time. However, after 9 weeks, the body weights in the L group slowly decreased. We speculated that the symptoms of diabetes increased slowly. At 13 weeks, the body weights in the L group were not significantly ($P > 0.05$) difference from those in the N group (Figure 2). This suggested that administration of the $L. casei$ CCFM0412 may prevent the body weight to excessively decrease in type 2 diabetic mice. However, Matsuzaki et al. [28] found that the body weights of $L. casei$-treated mice were lower than those of the control mice. This may have been due to the alteration of the host immune response by dietary restriction in that study [29].

We clearly demonstrated that administration of $L. casei$ CCFM0412 effectively reduced the fasting and postprandial 2-h blood glucose levels in mice (Figure 3). This may strengthen the hypothesis that $L. casei$ CCFM0412 can use glucose and change the intestinal environment. The inhibition of intestinal $\alpha$-glucosidase could postpone the digestion and absorption of carbohydrates and thus reduce postprandial hyperglycemia [30]. We demonstrated that $L. casei$ CCFM0412 showed better $\alpha$-glucosidase inhibitory activity in vitro (Journal of Chinese Institute of Food Science and Technology, accepted). HbA$_{1c}$ reflects the number of glucose molecules attached to hemoglobin in red blood cells and thus is an important factor used to monitor long-term blood glucose balance [12]. In the present study, administration of $L. casei$ CCFM0412 significantly lowered ($P < 0.05$) HbA$_{1c}$ values compared with the C group (Table 2). Yadav et al. [13] reported that in rats with induced type 2 diabetes, HbA$_{1c}$ levels in their dahi- and high fructose-treated groups were clearly lower than those in the high fructose-fed C group, which was consistent with our results. The liver, the most important organ in the regulation of glucose metabolism, assimilates
increased blood glucose in the form of glycogen [31]. In the present study, the liver
glycogen level was higher in the L group (Table 2) as the high load of blood glucose
was mobilized to and accumulated in the liver. Thus, the fasting and postprandial 2-h
blood glucose levels in the L group were significantly lowers ($P < 0.05$) than those in
the C group. This suggested that the blood glucose level of the high-fat-fed,
streptozotocin-induced type 2 diabetic mice administered with *L. casei* CCFM0412
might be controlled.

Impaired OGTT is an important criterion for type 2 diabetes [32]. A previous
study demonstrated that *L. rhamnosus* GG can improve glucose tolerance in
streptozotocin rats [12]. At 13 weeks, the increase in the level of glucose tolerance in
the L group was greater than that at 4 weeks (Figure 4). These results indicated that *L.
*casei* CCFM0412 could efficiently improve glucose tolerance in type 2 diabetic mice
as they grew over time. Similarly, *L. casei* CCFM0412 might prevent a decrease in
insulin secretion by protecting the islets of Langerhans (Table 2, Figure 6d). It was
clearly demonstrated that insulin secretion was increased and glucose tolerance was
unimpaired in streptozotocin-induced diabetic rats fed on LGG [12].

Endotoxin has been recognized as a novel factor triggering the onset of type 2
diabetes induced by a high-fat diet [33]. We observed that the L group showed a
significant decrease ($P < 0.05$) of endotoxin compared to values of C group (Figure
5A). This suggests that administration of *L. casei* CCFM0412 protected the mice
against endotoxin induced by type 2 diabetes. Cani et al. [34] found that endotoxin
positively correlated with glucose intolerance, consistent with our results. TNF-α is an
inflammatory cytokine that was identified as an important participant in insulin
resistance by inhibiting tyrosine kinase activity at the insulin receptor, which has been
suggested to play an important role in the development of type 2 diabetes [35]. In
contrast, IL-10 is an anti-inflammatory cytokine that inhibits antigen presentation and inflammatory cytokine production [36]. We found that L. casei CCFM0412 administered to high-fat-fed, streptozotocin-induced type 2 diabetic mice significantly lowered ($P < 0.05$) the value of TNF-α and increased the level of IL-10 (Figure 5B, C). This suggested that administration of L. casei CCFM0412 appeared to protect the mice against endotoxin and TNF-α induced by a high-fat diet.

Dyslipidemia is the common character in type 2 diabetes and is the primary cause of cardiovascular diseases in people with diabetes [18]. Previous studies have discovered that an increase in the production capacity of IL-10 will induce a gradual decrease in TG, TC, and LDL-C levels [5]. In this study, it was clearly demonstrated that the level of IL-10 increased and the values of TG, TC, and LDL-C decreased in the L group. In contrast, the level of HDL-C was significantly increased ($P < 0.05$) in the L group. The correlation between IL-10 levels and TNF and endotoxin concentrations and lipid and HbA1c parameters has been calculated by Pearson’s correlation test. The results showed that IL-10 negatively correlates with TNF-α ($r = -0.93$, $P = 0.07$), endotoxin ($r = -0.866$, $P = 0.134$), TG ($r = -0.958$, $P = 0.042$), TC ($r = -0.928$, $P = 0.072$), L-DLC ($r = -1$, $P = 0$) and HbA1c ($r = -0.931$, $P = 0.069$). Otherwise, IL-10 positively correlates with H-DLC ($r = 0.673$, $P = 0.327$). We suggest that the observed antidiabetic effect of L. casei CCFM0412 is probably attributable to its immunoregulatory properties.

The increased risk of dyslipidemia and chronically high blood glucose levels damage the antioxidant system of tissues [13]. Oxidative stress can impair the living cell membrane [37], and this may be the reason for the damage to the islets of Langerhans observed in our histopathological study. In the L groups, the islets of Langerhans were substantially protected from destruction compared with those in the
C group (Figure 6). Administration of *L. casei* CCFM0412 also resulted in an antioxidative effect in the liver tissue of the diabetic mice (Table 2). MDA is an end product and indicator of the lipid peroxidation process. GSH is thought to play an important role in the antioxidant defense system. Abnormal formation of ROS induced by a high glucose level may consume GSH rapidly and decrease GSH levels in the liver [39]. Many studies could not report a significant change in GSH-px activity between diabetics and controls [39, 40]. However, we observed a decrease in GSH-px activity in the C group compared with that in the N group. This finding is in accordance with that of Memisoğullari et al. [38]. Our results showed significant increase ($P < 0.05$) in GSH-px, GSH, and SOD levels and decrease in ROS and MDA levels in liver of the L group compared with those of the C group, which demonstrated that *L. casei* CCFM0412 appeared to have marked abilities to protect the liver against high blood glucose-induced oxidative damage.

Figure 7 presents the possible mechanism involved in antidiabetic effects of *L. casei* CCFM0412 on high-fat-fed and streptozotocin-induced type 2 diabetic mice.

**Conclusions**

This study demonstrated the antidiabetic effect of *L. casei* CCFM0412, which significantly improved glucose intolerance, dyslipidemia, immunoregulatory properties, and oxidative stress in high-fat-fed, streptozotocin-induced type 2 diabetic mice. Based on these results, we hypothesize that the mechanism of antidiabetic effect of *L. casei* CCFM0412 was inhibiting $\alpha$-glucosidase activity, improving immunoregulatory properties and oxidative stress. The results provide a sound rationale for future clinical trials of the oral administration of *L. casei* CCFM0412 for the primary prevention of type 2 diabetes.
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**Legends to Figures**

**Figure 1**

Experimental design

**Figure 2**

Change in body weight of mice administered *L. casei* CCFM0412 for 13 weeks. (■), N group; (●), C group; (▲), P group; (▼), L group. *P* < 0.05 compared with the value of C group.

**Figure 3**

Change in fasting (A) and postprandial 2 h (B) blood glucose levels of mice administered *L. casei* CCFM0412 for 13 weeks. (■), N group; (●), C group; (▲), P group; (▼), L group.* P* < 0.05 compared with the value of C group.

**Figure 4**

Increase in levels of blood glucose in mice as revealed by oral glucose tolerance tests after injected streptozotocin (A) (week 4) and before sacrifice (B) (week 13). AUC<sub>glucose</sub> are shown in the insets. (■), N group; (●), C group; (▲), P group; (▼), L group.

*<sup>a,b,c</sup>Values in the same column with different superscript letters significantly differ at* P < 0.05 by Tukey’s test.

**Figure 5**

The levels of endotoxin, TNF-α and IL-10 in blood serum of mice administered *L. casei* CCFM0412 at 13 weeks. (A) endotoxin; (B)TNF-α; (C) IL-10.
Values in the same column with different superscript letters significantly differ at $P < 0.05$ by Tukey’ test.

**Figure 6**

Representative photomicrographs of pancreatic sections of mice. (a) Pancreatic sections of mice in the N group; (b) Pancreatic sections of mice in the C group; (c) Pancreatic sections of mice in the P group; (d) Pancreatic sections of mice in the L group. H&E staining was used. Magnifications, $\times 200$.

**Figure 7**

Possible mechanism involved in antidiabetic effects of *L. casei* CCFM0412 on high-fat-fed and streptozotocin-induced type 2 diabetic mice.
Table 1 Composition of normal chow diet (A) and high fat diet (B)

(A)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Ingredients (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>9.20</td>
</tr>
<tr>
<td>Crude protein</td>
<td>22.10</td>
</tr>
<tr>
<td>Crude fat</td>
<td>5.10</td>
</tr>
<tr>
<td>Crude ash</td>
<td>5.20</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>4.12</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>50.00</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.24</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.92</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.34</td>
</tr>
<tr>
<td>Methionine and cystine</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Nutrient and energy composition

<table>
<thead>
<tr>
<th>Nutrient and energy composition</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloric ( Kcal/100g)</td>
<td>352.00</td>
</tr>
<tr>
<td>Carbohydrates (g/100 g)</td>
<td>52.00</td>
</tr>
<tr>
<td>Energy (%)</td>
<td>60.50</td>
</tr>
<tr>
<td>Proteins (g/100 g)</td>
<td>22.10</td>
</tr>
<tr>
<td>Energy (%)</td>
<td>25.70</td>
</tr>
<tr>
<td>Lipids (g/100 g)</td>
<td>5.28</td>
</tr>
<tr>
<td>Energy (%)</td>
<td>13.80</td>
</tr>
</tbody>
</table>
(B)

Constituents

<table>
<thead>
<tr>
<th>Ingredients (g/100 g)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal chow diet</td>
<td>54.70</td>
</tr>
<tr>
<td>Lard</td>
<td>16.90</td>
</tr>
<tr>
<td>Sugar</td>
<td>14.00</td>
</tr>
<tr>
<td>Casein</td>
<td>10.20</td>
</tr>
<tr>
<td>Premix</td>
<td>2.10</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>2.10</td>
</tr>
</tbody>
</table>

Nutrient and energy composition

<table>
<thead>
<tr>
<th>Nutrient</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloric (Kcal/100g)</td>
<td>445.50</td>
</tr>
<tr>
<td>Carbohydrates (g/100 g)</td>
<td>44.60</td>
</tr>
<tr>
<td>Energy (%)</td>
<td>40.00</td>
</tr>
<tr>
<td>Proteins (g/100 g)</td>
<td>22.30</td>
</tr>
<tr>
<td>Energy (%)</td>
<td>20.00</td>
</tr>
<tr>
<td>Lipids (g/100 g)</td>
<td>19.80</td>
</tr>
<tr>
<td>Energy (%)</td>
<td>40.00</td>
</tr>
</tbody>
</table>
Table 2 HbA1c, insulin, glycogen, lipid-metabolic, oxidative and antioxidative parameters in mice administered *Lactobacillus casei* CCFM0412 for 13 weeks.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>C</th>
<th>P</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (mmol/L)</td>
<td>8.10±0.20</td>
<td>12.37±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.97±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.56±0.50&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>2.64±0.12</td>
<td>1.55±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.72±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.25±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycogen (mg/g protein)</td>
<td>2.86±0.21</td>
<td>1.31±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.49±0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.40±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>13.39±1.08</td>
<td>15.32±1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.58±1.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.45±0.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>1.84±0.05</td>
<td>2.11±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.69±0.07&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.64±0.09&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>57.69±3.86</td>
<td>70.73±7.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.64±4.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.98±4.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>56.62±0.36</td>
<td>48.79±1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.91±1.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.14±0.40&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH-px (U/mg protein)</td>
<td>13.79±0.73</td>
<td>9.89±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.07±1.05</td>
<td>12.42±0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (mg/g protein)</td>
<td>12.47±0.11</td>
<td>10.62±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.24±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.48±0.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>75.25±2.47</td>
<td>48.77±3.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.04±3.29&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>69.53±1.41&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ROS (U/g protein)</td>
<td>13.24±1.01</td>
<td>14.86±1.46</td>
<td>13.25±1.35</td>
<td>12.04±0.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA (nmol/g protein)</td>
<td>65.35±2.26</td>
<td>87.42±3.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.07±3.45&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>69.80±2.82&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

N group, n=8; C group, n=8; P group, n=8; L group, n=8.

‘a’ (in C, P, L columns), significantly different from Normal group (*P* < 0.05); ‘b’ (in P, L columns only), significantly different from Control group (*P* < 0.05); ‘c’ (in L column only), significantly different from P group (*P* < 0.05).
weeks

1  3  4  13

N
normal chow diet + skim milk  

C
high-fat diet + skim milk  

P
high-fat diet + L. casei CCFM0412  

L

i.p. saline  

i.p. streptozotocin  

high-fat diet + pioglitazone  

high-fat diet + skim milk  

normal chow diet + skim milk  

normal chow diet  

high-fat diet
**L. casei**
CCFM0412

- Intestinal α-glucosidase activity
- Endotoxin TNF-α
- Antioxidant

- Blood glucose
- Inflammation
- Liver Islet cells

- Plasma lipids

Improve glucose tolerance
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