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Full Length Research Paper

Antioxidative and hypolipidemic effects of lactic acid bacteria from pickled Chinese cabbage

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Pickled cabbage is a popular Chinese traditional food. The present study was explored to characterize effects of lactic acid bacteria (LAB) isolated from the pickled cabbages on activities of antioxidant enzymes and hypolipidemia in normal and hyperlipidemic mice. 28 LAB strains were isolated from pickled cabbage, and two strains with high acid tolerance and bile salt resistance were screened. The strains were identified to be *Lactobacillus plantarum* (lab 1) and *Lactobacillus brevis* (lab 2) by the API 50CHL identification kit. They were given to normal and hyperlipidemic mice by ig.28 d continually. Activities of superoxide dismutase enzyme (SOD), glutathione peroxidase (GSH-px) in liver and kidney tissues of the LAB-treated mice were increased, while change in catalase (CAT) was insignificant. Differences of SOD levels between the lab 2-treated normal diet group and the normal control group was significant ($p<0.05$, $p<0.01$). Levels of serum total cholesterol (TC), total triglycerides (TG) and low-density lipoprotein cholesterol (LDL-c) were decreased and high-density lipoprotein cholesterol (HDL-c) level was higher in the LAB-treated groups. Compared with the high fat food control group, serum TC and TG levels were significant decrease ($p<0.01$), HDL-c level was significant increase ($p<0.05$) in lab 2-treated and lab 1+lab 2-treated high fat diet groups. However, the strains cannot decrease the blood glucose level of hyperglycemic mice. The result indicates that the strains have the potentials of enhancing activities of antioxidant enzymes and relieving hyperlipidemia-induced oxidative stress, which also suggested that Chinese pickled cabbage is the beneficial resources of probiotics.

Key words: Lactic acid bacteria, antioxidant effect, hypolipidemia, Chinese pickled cabbage.

INTRODUCTION

Hyperlipidemia is a risk factor for cardiovascular disease and the leading cause of death in many countries. Diet modification may be one way to reduce serum lipid level. Numerous studies have reported that lactic acid bacteria (LAB) fermented food display hypolipidemic effects by inhibiting cholesterol biosynthesis and decreasing low-density lipoproteins (Haberer et al., 2003; Kawase et al., 2000). *Momordica charantia* fermented milk is effective in preventing and retarding hyperlipidemia and atherosclerosis in hamsters (Tsai et al., 2009), and some kinds of LAB could adjust blood lipid and lower cholesterol, which can prevent and treat some diseases by activating antioxidant enzymes (Jain et al., 2009; Koïche and Dilmi, 2010). Probiotic LAB may promote the balance between microbial community and enzymes, and stimulate specificity and non-specificity immunity mechanism in body.

Meanwhile, it has some effects on accelerating

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Abbreviations: LAB, Lactic acid bacteria; **SOD,** superoxide dismutase; **GSH-px,** enzyme glutathione peroxidase; **CAT,** catalase; **TC,** total cholesterol; **TG,** total triglycerides; **LDL-c,** low-density lipoprotein cholesterol; **HDL-c,** high-density lipoprotein cholesterol.

development, strengthening physique, delaying aging and prolonging life (Guo, 2008). Recent researches demonstrate that humanity's senile might be associated with active free radicals, these free radicals may initiate a series of harmful biochemical response, thus can cause many kinds of illness occurrences such as cancer, diabetes, neurodegenerative and cardiovascular disease (Lee et al., 2008; Cave et al., 2006; Barnham et al., 2004).

The research demonstrated that cell-free extraction of LAB has some effects on antioxidation *in vitro* (Lee et al., 2005) and *in vivo* (Kaizu et al., 1993). Chinese pickled cabbage is a product made by the lactic acid fermentation, and the fermentation is manipulated by the salt concentration and temperature.

Fermented cabbages have made up a significant part of food intake in Asia countries for several centuries, including China, Japan, Korea, and so on. In previous studies, the chemical components and bacteriocin as biopreservatives of pickle usually were focused on (Huang et al., 2009; Zhao et al., 2007). In view of inactivation of gastric acid and bile salt for LAB, there are few researches on appraisement the probiotic functional properties of the living LAB from Chinese pickled cabbage *in vivo*.

In this study, 28 LAB strains were isolated from Chinese pickled cabbage, and two strains with high acid tolerance and bile salt resistance were screened, which were *Lactobacillus plantarum* and *Lactobacillus brevis*. The two strains were given to the normal, hyperlipidemic and hyperglycemic mice by ig.28d continually. Activities of antioxidant enzymes in liver and kidney tissues were observed, parameter of blood lipid was examined, and their effects of antioxidation and hypolipidemia were analyzed.

MATERIALS AND METHODS

Chemicals

The kits for superoxide dismutase enzyme (SOD), glutathione peroxidase (GSH-px), catalase (CAT), total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) were purchased from Nanjing Jiancheng Bioengineering Corporation (Nanjing, China). MRS broth was purchased from Beijing Biogenoro Biotechnology Co.LTD, China.

Strains isolated

LAB strains were isolated using MRS broth from Chinese pickled cabbage that was bought from the Shandongpu market, and identified according to Gram stain positive and catalase test negative. The isolated LAB strains were stored in -80℃.

Determination of acid tolerance

The 28 strains were grown in MRS broth at 37℃ overnight and subcultured in 10 mL of fresh MRS broth adjusted to pH 3 with hydrochloric acid (3.0 M). The initial bacterial concentration was 10^6 cfu mL^{-1} and was checked by viable count determination on MRS as described earlier. Samples were incubated for 4 h at 37℃. Cells were serially diluted 10-fold in phosphate buffer (0.1 M, pH 6.2) in order to neutralize the medium acidity. The residual viable count was determined by dilution and plate counting on MRS agar after 24 to 48 h of incubation. The survival rate was calculated as the percentage of colonies grown on MRS agar compared to the initial bacterial concentration.

Bile salt tolerance test

MRS broth was inoculated with 10^6 cfu mL^{-1} from overnight cultures. Test cultures were supplemented with 0.3% oxgall (Sigma Chemical Co., St. Louis, MO USA). Growth in control (no bile) and test cultures (0.3% oxgall) was monitored and incubated for 4 h at 37℃. Cells were serially diluted 10-fold in phosphate buffer (0.1 M, pH 6.2) in order to dilute the medium bile salt. The residual viable count was determined by dilution and plate counting on MRS agar after 24 to 48 h of incubation. The survival rate was calculated as the percentage of colonies grown on MRS agar compared to the initial bacterial concentration.

From acid and bile salt tolerance tests, two LAB strains showed high acid tolerance and bile salt resistance. The strains were identified to be *L. plantarum* (lab 1) and *L. brevis* (lab 2) by the API 50CHL identification kit (BioMerieux SA, France), and stored in the Bioengineering laboratory of Yanshan University (Qinhuangdao, China).

Experimental design *in vivo*

Male ICR mice weighing 18 to 22 g were purchased from the Experimental Animal Center of China Academy of Military Medical Science (Beijing, China). 56 mice were divided randomly into 7 groups:

Normal control group (NC), high fat control group (HFC), lab 1 + normal diet group (lab 1 + ND), lab 1 + high fat diet group (lab 1 + HFD), lab 2 + normal diet group (lab 2 + N D), lab 2 + high fat diet group (lab 2 + HFD) and mixed bacteria + high fat diet group (MB + HFD). The mice of the HFC, lab 1 + HFD, lab 2 + HFD and MB + HFD groups were fed with high fat diet 30 d continually to construct hyperlipidemic models.

On the day 31, the living lab 1 and lab 2 suspensions were fed to the mice by ig. 28 d continually. Experiment groups and feeding treatments are shown in Table 1.

Determination of tissue enzyme activities and biochemical metabolic parameters

On the day 59, the mice were killed under ether anesthesia, and blood samples were collected from eye pit of all the mice. Livers and kidneys of the mice were removed immediately, weighed and washed with cold physiological saline, and the 10% tissue homogenate was prepared using physiological saline by a cold glass homogenizer. The homogenate was centrifuged at 4000 × g for 15 min at 4℃, and the supernatant was stored at -80℃ for further analysis.

The protein concentration of the tissues supernatant was determined by the DC Protein Assay Kit (Bio-Rad Labratories; Richmond, CA) based on the Lowry colorimetric assay (Lowry et al., 1951) using bovine serium albumin as standard. The levels of CAT, GSH-px, SOD, TC, TG, HDL-c and LDL-c were measured according to the commercial instructions.

Table 1. Experiment groups and ways of feeding.

Group	Dosage(Ml), Bacteria number (cfu mL^{-1})	Experimental animal quantity	Feeding method
NC	0.2, 2×10^9	8	Normal diet
HFC	0.2, 2×10^9	8	High fat diet
Lab1 + ND	0.2, 2×10^9	8	Lab 1 suspension gavage, normal diet
Lab1 + HFD	0.2, 2×10^9	8	Lab 1 suspension gavage, high fat diet
Lab2 + ND	0.2, 2×10^9	8	Lab 2 suspension gavage, normal diet
Lab2 + HFD	0.2, 2×10^9	8	Lab 2 suspension gavage, high fat diet
MB + HFD	0.2, 2×10^9	8	LLb 1+lab 2 suspension gavage, high fat diet

The values are mean ± SD.*p <0.05 vs normal control group. **p <0.01 vs normal control group.$^+$ p<0.05 vs high fat control group. $^{++}$ p<0.01 vs. high fat control group.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 software. Data are expressed as mean with SD. Differences in antioxidant enzymes and biochemical parameters among control and LAB-treated groups were analyzed using one-way ANOVA, followed by the Scheffe test. The result was considered significantly different at level of $p < 0.05$.

RESULTS

Effects of LAB on SOD activities of the experimental mice

SOD plays a very important role in the balance between oxidation and antioxidation. It could eliminate superoxide free radicals and protect body cells against superoxide damage. Figure 1 indicates that the decrease in the level of SOD was significant in the HFC group compared with NC group (p<0.05). The levels of SOD were increased in lab 1 + ND and lab 2 + ND groups compared with NC group, but were not significant. However, the activities of SOD were significant in liver and kidney tissues of lab 1 + ND and lab 2 + ND groups compared with HFC group (p<0.01). Meanwhile, the activities of SOD in lab 1 + HFD, lab 2 + HFD and MB + HFD groups in kidney tissues were increased significantly compared with HFC group (p<0.05), and the levels of SOD in liver tissue were enhanced in lab 2 + HFD and MB + HFD groups than that of HFC group (p <0.05).

Effects of LAB on GSH-px and CAT activities on the tested mice

GSH-px is a key antioxidant enzyme catalyzing the reduction of peroxides to protect against oxidative tissue damage. Figure 2 shows effects of lactic acid bacteria on tissue GSH-px activities which demonstrate that there was significant difference between NC and HFC groups (p<0.01). GSH-px activities in liver and kidney tissues of lab 1 + ND group has no significant difference compared with NC group, but the lab 2 + ND group was significantly different compared to NC group (p<0.05). GSH-px levels in the lab 1 + HFD, lab 2 + HFD and MB + HFD groups were different significantly compared with HFC group (p<0.05, 0.01).

CAT is a main enzyme in the microbody of cells, which can oxygenolysis toxic components. The activities of CAT of liver and kidney tissues for the HFC group were different significantly compared with NC group (p<0.05). However, difference of the levels of CAT was insignificant between all LAB-treated groups and NC group (p>0.05, Table 2). The result indicated that the two strains may have no effect on the CAT activities for the LAB-treated mice.

Effects of LAB on serum lipid parameters of tested mice

The results of TC and TG levels of the experimental mice are presented in Table 3. The levels of TC and TG in lab 1 + ND and lab 2 + ND groups were slightly lower, but which were not significant compared with NC group. TC and TG levels of hyperlipidemic mice were higher than NC group, and difference was extremely significant (p<0.01), which indicated that the hyperlipidemic models were established. TC and TG levels in lab 1 + HFD, lab 2 + HFD and MB + HFD groups were significantly lower compared to HFC group (p<0.01), and the MB + HFD group was the lowest, which may be contributed to the mixture of the two strains. The result showed that the two strains have good effects on cholesterol-degrading activity.

LDL-c is a main factor of the danger on atherosclerosis, and HDL-c plays an important role in protecting cardiovascular system. HDL-c levels in the LAB-treated hyperlipidemic groups were all higher than HFC group (p<0.05 with lab 2 + HFD group and p <0.01 with MB +

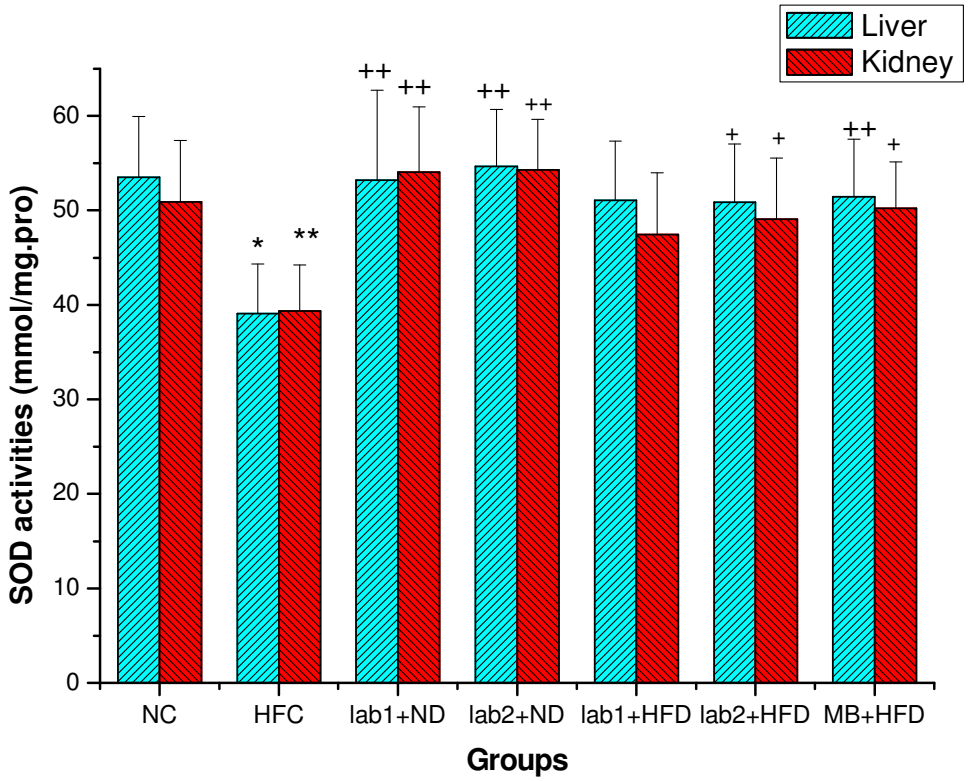

Figure 1. Effects of lactic acid bacteria on tissue SOD activities. The values are mean ± SD.*p<0.05 vs normal control group (NC). **p<0.01 vs NC group. ^{+}p<0.05 vs high fat control group (HFC), ^{++}p<0.01 vs high fat control group.

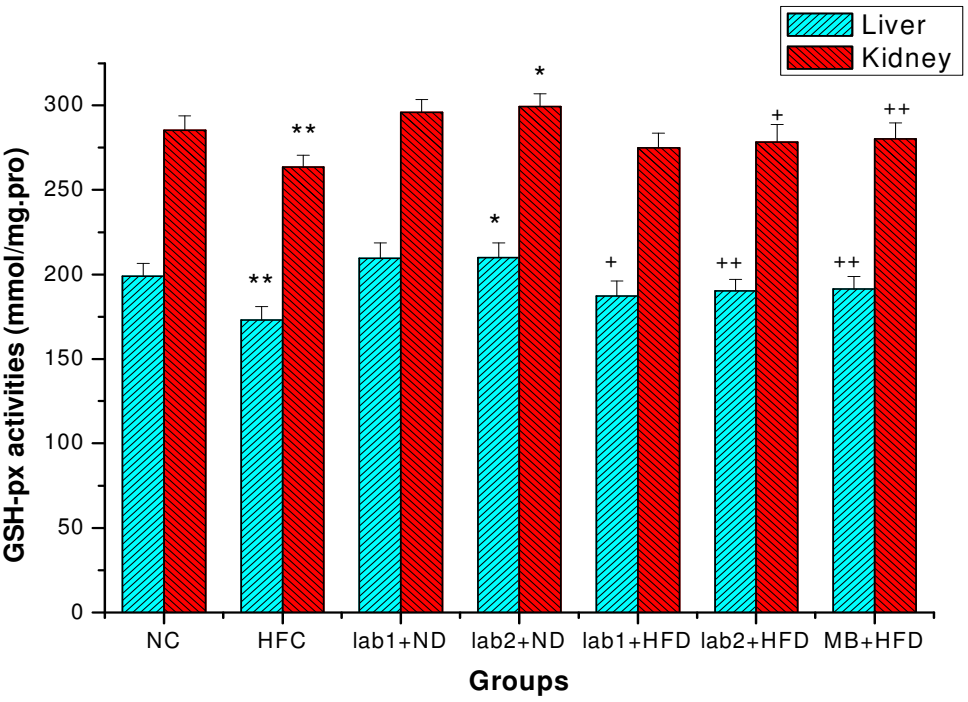

Figure 2. Effects of lactic acid bacteria on tissue GSH-px activities. The values are mean ± SD. *p<0.05 vs normal control group. **p<0.01 vs normal control group. ^{+}p<0.05 vs high fat control group (HFC), ^{++}p<0.01 vs high fat control group.

Table 2. Effects of lactic acid bacteria on tissue CAT activities of the tested mice.

Group	CAT (mmol/mg.pro)	
	Liver	Kidney
NC	1.08 ± 0.18	1.07 ± 0.24
HFC	0.79 ± 0.15*	0.85 ± 0.15*
Lab 1 + ND	0.92 ± 0.17	1.01 ± 0.21
Lab 2 + ND	0.94 ± 0.14	0.99 ± 0.26
Lab 1 + HFD	0.97 ± 0.14	0.94 ± 0.14
Lab 2 + HFD	0.98 ± 0.12	0.99 ± 0.21
MB + HFD	0.93 ± 0.15	1.01 ± 0.27

The values are mean ± SD. *p<0.05 vs normal control group.

Table 3. Effects of lactic acid bacteria on blood lipid levels.

Group	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)
NC	2.76 ± 0.11	2.35 ± 0.25	1.88 ± 0.18	3.81 ± 0.18
HFC	9.03 ± 0.05**	5.21 ± 0.08**	4.81 ± 0.02**	4.99 ± 0.18*
lab1 + ND	2.58 ± 0.16^{++}	2.27 ± 0.15^{++}	2.01 ± 0.16	3.37 ± 0.16
lab2 + ND	2.674 ± 0.15^{++}	2.31 ± 0.24^{++}	2.16 ± 0.10	3.45 ± 0.19
lab1 + HFD	7.12 ± 0.14	3.86 ± 0.10	4.98 ± 0.14	4.19 ± 0.26
lab2 + HFD	6.59 ± 0.04^{++}	3.25 ± 0.04^{++}	6.22 ± 0.14^{+}	3.93 ± 0.32^{+}
MB + HFD	6.06 ± 0.09^{++}	3.02 ± 0.01^{++}	7.74 ± 0.03^{++}	3.71 ± 0.19^{+}

HFD), and levels of LDL-c were lower significantly in lab 2 + HFD and MB + HFD groups compared with that of high fat control group (p<0.05, Table 3).

Effect of LAB on blood glucose levels of the experimental mice

The blood glucose level was not significantly changed when the experimental mice were treated using the living LAB suspensions for 28 d. But in the high fat food fed mice, the blood glucose level increased obviously and even exceeded the threshold value of fasting plasma glucose (6.1 mmol/L). The results revealed that high lipid food could be correlated with diabetes, which also reminded us that low-fat food may be reducing the hyperglycemic risk. When the high fat diet groups were given the living LAB bacteria suspensions, there was no significant difference between the LAB-treated mice and the HFC mice (p>0.05). Therefore we infer that the two strains may have no hypoglycemic effect for the diabetic mice (Figure 3).

DISCUSSION

Probiotics are commonly used as viable microbial feed supplements that affect the host animal by improving its intestinal microbial “balance”. Human gastrointestinal tract is adapted more or less to a daily supply of live lactic acid bacteria (Holzapfel et al., 1998). Several studies demonstrated that some lactobacilli possess antioxidative activity, and could decrease the risk of accumulation of reactive oxygen species during the ingestion of food (Ito et al., 2003; Kuda et al., 2010). It also indicated that lactobacillus ferment was a functional material which having antiobesity and antimicrobial effects (Herreros et al., 2005; Gonzalez et al., 2007). However, probiotic bacteria must be resistant to the acidity of the stomach, lysozyme, bile, pancreatic enzymes. High acidity in the stomach and high concentration of bile components in the proximal intestine are the first host factors, which affect strain selection and adhesion. In present study, 28 LAB strains were isolated from Chinese pickled cabbage, and two high acid tolerance and bile salt resistance strains were screened, which were *L. plantarum* and *L. brevis*, respectively.

Activities of antioxidant enzymes and the function of reducing cholesterol and blood glucose of the 2 LAB strains were studied. The experimental mice were divided into normal diet and high fat diet groups. The concentration of lipids in the blood of normal diet mice was under normal range. When the mice have been fed high fat food for 30 d in the high fat diet group, the levels

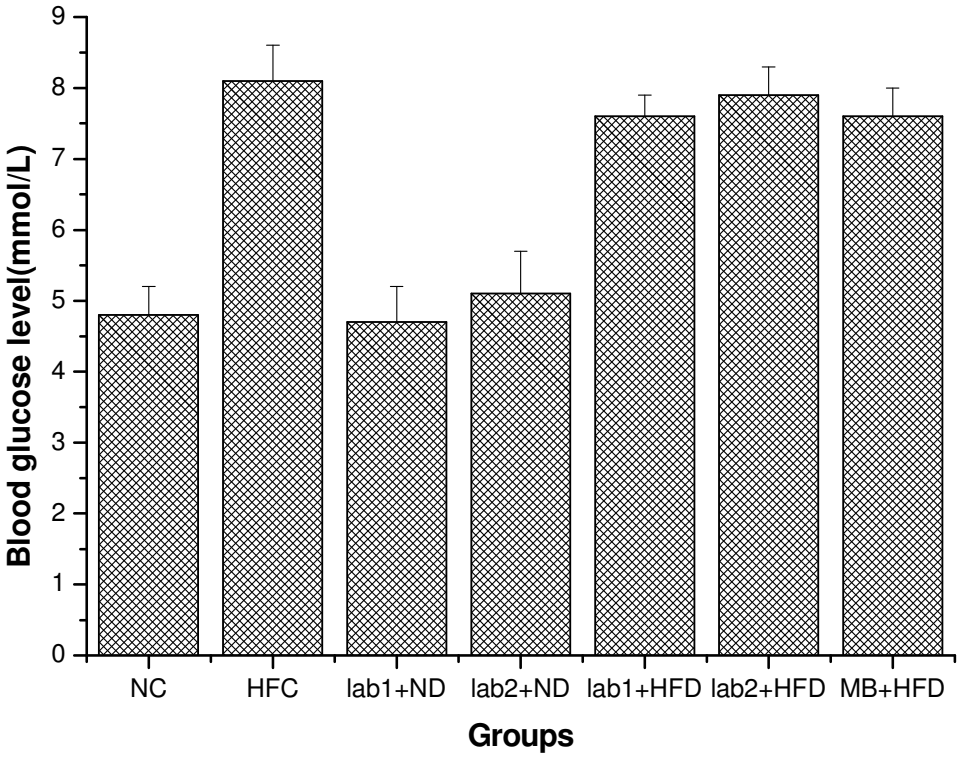

Figure 3. Effect of lactic acid bacteria on blood glucose levels of the experimental mice. The values are mean ± SD.

of TC, TG and glucose in the blood were higher than those of normal diet mice, respectively. Levels of serum TC, TG and LDL-c were slightly decreased and HDL-c level was a little higher by the LAB suspension treating on the normal mice. However, compared to the HFC group, levels of TC and TG were decreased extremely in lab 2 + HFD and MB + HFD groups. The findings indicates that the two strains might decrease the risks for cardiovascular and arteriosclerosis diseases in various degrees, and it also could fall significantly the cholesterol level in hyperlipidemic mice. But the strains have little effect on serum lipid of the normal diet mice.

Akalin et al. (1997) found that consumption of acidophilus yogurt significantly lowered the values for plasma TC, LDL-c in the mice. After the male SD rats were fed high-fat diet with *Lactobacillus* ferment, there were significantly decrease on the levels of body weight, LDL-c and TC compared with the high-fat diet control rats (Choi et al., 2006). Researchers showed that LAB could decrease the level of cholesterol but the mechanism has not been demonstrated clearly yet. Some predicted that LAB, bile salt and cholesterol were coprecipitated, and then expelled with feces, or the cholesterol was absorpted by lactic acid bacteria (Jeun et al., 2010). Otherwise, Smet et al. (1994) suggested that the reason of LAB reducing cholesterol might be due to the activity of bile salt hydrolysis produced by the LAB. Further research is indispensable to clarify the exact mechanism.

Several studies have documented the relationships between increase of free radicals and blood glucose, lipid peroxidation as well as low-density lipoprotein (Tanaka et al., 2002). Free radicals can diffuse intracellularly and result in mitochondrial enzyme damage and DNA breaks, impair cellular function (Bonnefont-Rousselot et al., 2000). SOD is a scavenger of free radicals, which has important effects on control of oxidation reactions in the body. Some LAB may enhance the SOD and GSH-px activities and prevent oxidative damage (Tsai et al., 2009). GSH is often regarded as an antioxidant agent, since it protects protein –SH groups against oxidation and can scavenge oxygen radicals and some other reactive species. It reduces different oxidants after increasing its hydrogen atom. This reaction is catalysed by enzyme GSH-px in cells (Reiter, 1995).

In the present research, the concentration of SOD and GSH-px in the high fat diet mice was significantly lower than those of the normal rats. Meanwhile, the activities of SOD and GSH-px were increased in various degrees in lab 1 + ND and lab 2 + ND groups compared with the normal control mice after the LAB administering. The levels of SOD and GSH-px in the lab 1 + HFD, lab 2 + HFD and MB + HFD groups were all increased compared with HFC group, but not achieve the level of the NC group. This suggests that the two strains from the Chinese

pickled cabbage have effective antioxidative properties and could scavenge well excess free radicals. In this study, the levels of antioxidant enzymes and the function of reducing blood lipid in MB + HFD groups were higher than the other two hyperlipidemic groups, which may be attributed to the synergetic effect of the two strains.

This research found that there was no significant difference on CAT activities in the LAB-treated groups. Some kinds of LAB have an antioxidant system of NADH oxidase/NADH peroxidase (Lountos et al., 2006). However, the activity of NADH peroxidase in some LAB strains was low and could not reduce the toxicity of H_2O_2 (Chang et al., 1998). The results of this experiment may be correlated to the metabolism. Recent investigations have revealed that there is a correlated relationship between hyperlipidemia and diabetes mellitus. It considered that long-term consumption of high fat food may induce disorder of glucose and lipid metabolism. It also could induce insulin sensitivity descending and insulin resistance occurring (Greenwood et al., 2005). In our study, when the mice were fed with the high fat diet for 30 d, their fasting plasma glucose were increased and even exceeded the threshold value, which imply that high fat food may induce the occurrence of hyperglycemia. However, there was not marked change on the plasma glucose of the hyperlipidemic mice after they were given the living LAB suspensions 28 d.

In conclusion, *L. plantarum* and *L. brevis* isolated from the Chinese pickles cabbage demonstrated potential for cholesterol-lowering and antioxidation. Additionally, a LAB management in diet might be an effective approach to control the lipid metabolism. Furthermore, the Chinese pickle cabbage might be a valuable source of probiotics, therefore further genetic and functional studies should be performed to characterize in depth the indigenous isolates.

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