具有胃肠道吸附能力及改善脂质代谢效果的植物乳杆菌BB9

Lactobacillus plantarum BB9 is capable of adhering to gastrointestinal tract and to improve the lipid metabolism

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Abstract

本研究主要为评估本研究主要为揭示植物乳酸杆菌BB9对肠道细胞的吸附能力及改善血液脂质的效果。BB9菌株系分离自台湾的水果，实验证实具有耐酸和耐胆盐性质，且对肠道细胞具有非常优异的吸附能力。实验以高热量、高胆固醇饮食喂食仓鼠以诱发高胆固醇血症及肥胖，同时投与不同剂量的BB9乳酸菌，结果证实BB9菌株可有效降低肝脏及血液中的胆固醇、三酸甘油脂及LDL，并且可降低仓鼠的体重，此外，粪便中BB9菌株的数量也显著增加。因此，此植物乳酸杆菌BB9菌株可预防人类或动物之心血管疾病与肥胖，可应用于药物、健康食品、食品和饮料等。

The objective of this study was to evaluate the effects of *Lactobacillus plantarum* BB9 on adhering ability to gastrointestinal cell and cholesterol-lowering effects. This strain may apply in the fields of pharmaceuticals, health food, weight loss diet, health supplement products, food and beverages. *L. plantarum* BB9 was isolated from fruits in Taiwan. In-vitro tests show that this strain has acid and bile tolerance, as well as strong adhering ability to gastrointestinal cells. In-vivo study, hamsters were divided into five groups and fed with high-fat diet to induce hypercholesterolemia. The groups include fed with different dose of *L. plantarum* BB9, and *L. acidophilus* ATCC 43121 as positive control. Results show that fed with low or high dose of *L. plantarum* BB9 has reduced the levels of cholesterol and triglycerides (TG) in blood and liver effectively and better than the control group. Meanwhile, the HDL-c/LDL-c ratio is also improved significantly. In the monitoring of body weight, it is found the weight gained in fed with *L. plantarum* BB9 group is less than those of the other fed with high-fat diet groups. In addition, the viable count of *L. plantarum* BB9 is increase significantly in feces. Therefore, *L. plantarum* BB9 may provide the beneficial function in the prevention or treatment of cardiovascular diseases in animals and humans.

Introduction

High blood lipid level is one of the major causes of cardiovascular disease and atherosclerosis. The levels of triglycerides (TG), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) are also highly associated with cardiovascular disease. Clinically, blood cholesterol level is a good predictor of the risk of cardiovascular diseases. Lactobacilli are commonly existed in the intestinal microflora of humans and animals and offer health benefits. Thus they are often used as probiotics and are added in food products for health enhancement. Mann and Spoerry observed in 1974 that the concentration in the blood has lowered after consumption of large amount of lactobacillus-fermented milk. Since then, the correlation between Lactobacillus and cholesterol has become a research topic. So far, the mechanism of how probiotic Lactobacillus lowers cholesterol has not yet been clearly discovered. Strain of Lactobacillus acidophilus ATCC 43121 has been tested and proved to lower Hypercholesterolemia. Similarly, this study also develop a new probiotic that is safe and efficacy to help the patients to control the risks and clinical symptoms of cardiovascular diseases.

Materials and Methods

(1) Acid and bile salt tolerance

For acid tolerance, each lactic acid bacteria (LAB) culture was added into 9 ml phosphate saline buffer (PBS) which had been adjusted to different pH values (pH2.0, 2.5, 3.2 and 7.2). Each mixture was incubated at 37˚C for 3 h and then the viable counts were determined.

For bile salt tolerance, aliquots of 0.1 ml LAB suspension was added to 9.9 ml with or without 0.3 % ox gall MRS broth and incubated at 37˚C under anaerobic conditions. Tolerance to bile was determined by measuring OD600 nm during incubation and calculating the ratio of growth rate.

(2) Adhesion of LAB to intestinal cell lines

Viable LAB strains were added to each well and incubated for 1 h at 37˚C in a CO_2 incubator. LAB were adjusted to 1×10^8 cfu/ml. After incubation, cells in each well were washed with PBS, fixed and stained. The adhering LAB were visually counted using microscopy (200X). The number of adherent LAB per Caco-2 cell was calculated by counting LAB cells on about 100 cells in 15 randomly selected microscopic.

(3) **In-vivo animal testing**

The hamsters were randomly divided into 5 groups and 6 hamsters per group. The feed formulation was based on AIN-76 and further included the ingredients of casein, cornstarch, soybean oil, fiber, mineral mix, vitamin mixture, sucrose, cholesterol and cholic acid. Except for the blank control group, the feeds for the other groups had the proportion of soybean oil increased to 15% and 0.5% cholesterol added to induce hypercholesterolemia. The feeds for the test groups were added with freeze-dried LAB in powder form. All feeds were stored at 4℃. Fresh feed and sterile RO water were provided daily.

Grouping —

A : Naïve group – fed with AIN-93 without cholesterol or LAB powder.

B : High-Fat group – fed with high fat and 0.5% cholesterol.

C : High Fat feed ＋ 1×10^9 CFU/g L. acidophilus ATCC 43121 (ATCC 43121-9') – fed with high fat and 0.5% cholesterol, and containing freeze-dried powder of ATCC 43121 strain by final concentration of 1×10^9 CFU/g.

D : High Fat feed ＋ 1×10^9 CFU/g L. plantarum BB9 (BB9-9') – fed with high fat and 0.5% cholesterol, and containing freeze-dried powder of BB9 strain by final concentration of 1×10^9 CFU/g.

E : High Fat feed ＋ 1×10^{10} CFU/g L. plantarum BB9 (BB9-10') – fed with high fat and 0.5% cholesterol, and containing freeze-dried powder of BB9 strain by final concentration of 1×10^{10} CFU/g.

Assay—

The body weights of hamsters were recorded every week. After four weeks, the hamsters were sacrificed. Their plasma and liver were collected and then stored at 4℃. Finally, the total cholesterol, TG, HDL, LDL and TNF-alpha were assayed. In addition, the number of BB9 was counted in faeces.

Results

(1) Acid and bile salt tolerance

Results showed that QK7 and BB9 had better tolerance at pH 2.0 than the other strains, and the survival rates were greater than 5-log units. In addition, viable counts at pH 2.5 were higher than at pH 2.0. There were no obvious differences in tolerance or survival among all bacterial strains at pH 3.2. For bile tolerance, results showed that BB9 had higher bile tolerance than other strains. (Table 1)

(2) Adhesion ability

The results were showed in Table 2. Only *L. plantarum* BB9 had strong adhesion ability.

(3) Weight change

As showed in Table 3. The BB9 groups had gained the lowest body weight than the other groups. In addition, it may have dose-dependent effect.

(4) Lipid assay of plasma and liver

Most of the livers of sacrificed hamsters fed with high fat and cholesterol diets showed white adipose tissue, indicating serious pathology of fatty liver. In plasma lipid analysis, their total cholesterol and TG were significantly higher ($P<0.05$) as compared to naive group (FIG. 1). But hamsters fed with freeze-dried BB9 strain showed lower cholesterol, TG and LDL-cholesterol ($P<0.05$). When fed with high dose of 1×10^{10} CFU/g BB9 strain that showed the best lowering effect (FIG. 1 & FIG. 2). FIG. 3 indicated the analysis of cholesterol and TG in liver. The results were almost the same with the results of plasma. The positive effect of BB9 strain have dose-dependent response.

(5) Viable count of BB9 in faeces

When fed with BB9 strain, the viable count of LAB in faeces was increased significantly ($P<0.05$). Also, it have dose-dependent response. Furthermore, the increased LAB were proved by PCR that BB9 strain was major bacteria.

Table 1-Analysis of acid and bile tolerance of tested LAB strains.

Strains	Acid tolerance (log cfu/ml)†				Bile tolerance (%)‡
	0 hr	3 hr			
	pH 7.0	pH 2.0	pH 2.5	pH 3.2	
AN36	9.03±0.01	3.49±0.21	4.84±0.16	8.81±0.05	30.1
QK7	8.33±0.11	7.01±0.15	7.42±0.18	7.80±0.06	15.3
LA51	7.87±0.02	2.95±0.16	4.50±0.09	7.75±0.06	7.5
FY5	8.45±0.07	4.19±0.13	5.42±0.07	8.30±0.02	23.5
P33	7.68±0.03	3.51±0.10	4.55±0.08	6.68±0.08	70.4
AB1	7.55±0.06	3.35±0.11	4.26±0.09	6.24±0.10	45.6
BB9	**9.03±0.11**	**5.49±0.19**	**6.84±0.09**	**8.81±0.05**	**57.9**
TS6	9.04±0.05	4.68±0.02	5.53±0.03	8.78±0.07	41.0

Table 2-Adherent activity of LAB to the Caco-2 cells

Strains	adherent LAB per cell (cfu/cell)†
AN36	0
QK7	0
LA51	0
FY5	6.1±1.8
P33	0
AB1	0
BB9	25.1±2.2
TS6	0

FIG. 1

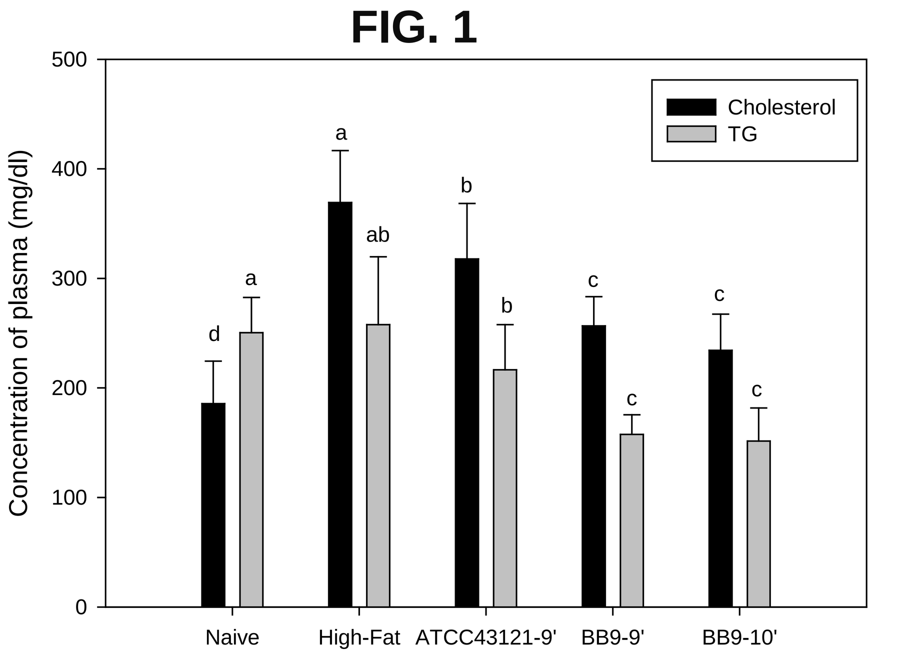

Table 3-Weight change between experiment periods.

	1 week	after 4 weeks	Weight change
Naive	107.2	126.3	19.1
High-Fat	106.7	132.8	26.1
ATCC43121-9'	108.9	126.5	17.6
BB9-9'	106.8	122.3	15.6
BB9-10'	108.2	120.8	12.6

FIG. 2

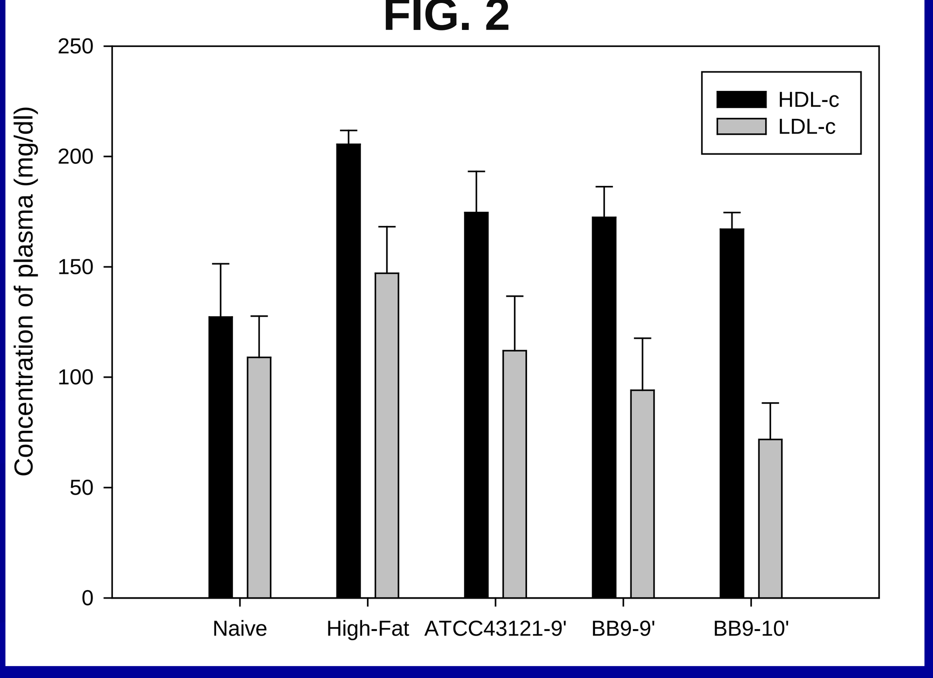

FIG. 3