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Bile Acid Metabolism in Hyperlipidemia Mice Effects of Lipid-Lowering Lactobacillus Plantarum DC4

Jiajing Song, Siying Wu, Lingzhu Zeng, Wei Xu, Yi Tian, Ziqi Wu, Lei Zhu, Jiantao Liu*

School of Life Sciences, Jiangxi Science & Technology Normal University, Nanchang, Jiangxi, 330013, China

**Corresponding author*

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Abstract: The purpose of this experiment was to study the changes of bile acid metabolism during the lipid-lowering process of Lactobacillus plantarum DC4. To analyze the body effect of Lactobacillus plantarum DC4 regulating cholesterol metabolism, and to explore the metabolic network pathways it participates in or regulates, and to preliminarily elucidate its mechanism of regulating cholesterol metabolism. Taking the intervention of bile acid metabolism network as the starting point, the effects of Lactobacillus plantarum DC4 on abnormal lipid metabolism and bile acid profile in hyperlipidemia mice were investigated. Combined with QPCR technology, the metabolomics characteristics of bile acids before, during and after Lactobacillus plantarum DC4 intervention were studied to find related and co-changed metabolites and influencing pathways, and to elucidate the mechanism of Lactobacillus plantarum DC4 improving lipid metabolism in hyperlipidemia mice.

1. Introduction

Lactobacillus plantarum is a Gram-positive anaerobic or facultative anaerobic Lactobacillus, which is widely distributed in nature, especially in various fermented foods, dairy products, fermented dough and so on [1]. The optimum temperature range is generally 30 °C to 35 °C. The optimum pH is 6.5. The colonies of Lactobacillus plantarum are usually white, round, smooth surface and different shapes. The species of Lactobacillus plantarum DC4 are non-spore-forming bacteria. The probiotic is a homofermentative lactic acid bacteria. It is common in the mouth, intestines and other parts of animals. It is a kind of beneficial probiotics in the human gastrointestinal tract, which can promote the effective metabolism of many natural antibacterial substances, such as hydrogen peroxide, organic acid, diacetyl and bacteriocin.

Lactobacillus can regulate and maintain the function of gastrointestinal flora [2]. It can increase the number of beneficial bacteria in the human gastrointestinal tract, reduce the number of harmful bacteria, keep the microbial content within the normal range, and maintain the normal physiological state of the gastrointestinal environment. Lactobacillus plantarum can not only reduce cholesterol content, but also lower blood pressure and blood lipids [3].

It can also protect against cardiovascular disease by increasing the production of nitric oxide (a natural vasodilator), reducing inflammation and oxidative stress. It also has certain pharmacological effects in clinical practice, such as improving constipation and diarrhea. In addition, Lactobacillus can also improve the Th1 / Th2 balance, enhance NK activity, and inhibit the reduction of human immunity. It can effectively protect the human immune system from being affected. Studies have found that it can also effectively treat the symptoms of lactose intolerance, promote the absorption of nutrients, reduce the absorption of cholesterol, control blood lipid and blood pressure index, maintain liver and increase liver detoxification and other probiotic functions [4]. In recent years, researchers are further exploring the mechanism of its efficacy.

2. Materials and Methods

2.1. Experimental Organisms

Male mice, Lactobacillus plantarum DC4, Escherichia coli, Staphylococcus, Salmonella typhi, Shigella Flexner, Bacillus cereus, Pseudomonas aeruginosa.

2.2. Instrument and Equipment

Ultra-clean bench, electronic scale, pipette gun, slides, water bath, sampler, washing machine, planning knife, tweezers, glycerin, fluorescence quantitative PCR instrument, measuring cylinder, volumetric flask, microplate reader, burette, Columbia blood agar medium, incubator, antibiotics, paper, puncher, ultraviolet spectrophotometer, MRS medium.

2.3. Main Reagents

Hematoxylin dye, ammonium hydroxide, eosin dye, simulated gastric acid, simulated intestinal fluid, 0.3 % bile salt solution.

2.4. Experimental Method

2.4.1. Effect of Lactobacillus Plantarum DC4 on Bile Acid Metabolism in Hyperlipidemia Mice

Firstly, the bile acid spectrum of fecal samples of mice in each group was detected. Using the method of batch detection, the feces of mice were sampled three times before and after. The fecal content of each sampling was the same, and the extraction post-processing method was the same. UPLIFTS was used to quantitatively detect 61 bile acids. The bile acids of mice in each group and the contents of different kinds of bile acids in the group were marked respectively, and the bile acid spectrum chart was drawn [5].

2.4.2. Effects of Lactobacillus Plantarum DC4 on Liver and Blood Lipid in Hyperlipidemia Mice

(1) Detection of the effect of Lactobacillus plantarum DC4 on liver and blood lipid lipid content in hyperlipidemic mice

The mice were randomly assigned into an equal number of standard control groups as well as experimental groups (hyperlipidemic rats). The standard control group was fed a normal diet and the experimental group was fed a high-fat diet. After four weeks of modeling, the rats were tested for lipid parameters, and rats with hyperlipidemia were cultured and then screened for successful modeling [6]. The experimental animals continued to be randomly divided equally into 3 groups according to parameters such as serum TC, LDL-C levels and body weight, and were given the corresponding drugs by gavage, labeled as model group, ezetimibe group, and Lactobacillus plantarum DC4 group. The changes of TC, TG, total bile acids and LDL-C levels in serum and liver of rats in each group were recorded according to the enzyme chemistry assay by hematoxylin-eosin (HE) staining method [7]. The changes of liver-related indices of rats in each group were detected and observed. The isolated sera were subjected to biotin double antibody sandwich enzyme-linked immune dependent assay (ELISA). To detect serum lipocalin (ADP)) levels. One replicate well was set up for each sample, and the graph was plotted according to the instructions of the Mouse ADP ELISA kit [8].

(2) To examine the regulation of key genes of bile acid metabolism in hyperlipidemic mice by Lactobacillus plantarum DC4

Each group of experimental mice was fed the appropriate chow in the manner described above. At the end of the 8th week of the experiment, each group of mice was given the appropriate chow to feed, and after blood collection, the animals were executed by cervical dislocation, and the livers were quickly stripped and stored at -80 °C for cryopreservation, and the process should be continuous. About 6 cm of small intestine tissue was quickly excised from 2 cm below the stomach and stored at -80°C to detect the expression of relevant genes in the small intestine of mice [9].

3. Results and Analysis

3.1. Cholesterol Removal Rate of Lactobacillus Plantarum DC4 in Vitro

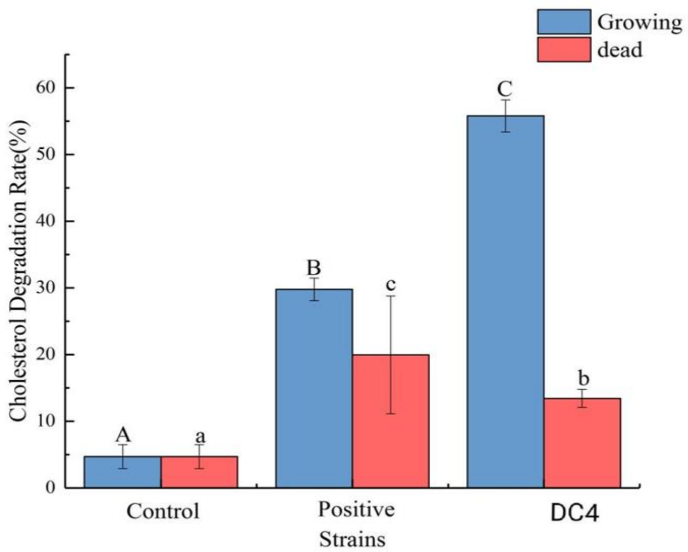

Figure 1: Vitro cholesterol removal rate of Lactobacillus plantarum DC4

To determine the in vitro cholesterol-lowering rate of Bacteroides DC4, the first step is to perform cholesterol-lowering experiments in vitro on the organism [10]. Preliminary analysis of whether the experimental strains have potential lipid-lowering properties based on in vitro cholesterol-lowering correlation coefficients. The effect of bacteriophage DC4 on the cholesterol content was determined using the o-phthalaldehyde method. According to the results shown in Figure 1, after 24 h of incubation, the Live cells of the bacterium DC4 and heating to dead cells in vitro cholesterol removal rates were (55.79±2.4%) and (13.42±1.35%, respectively) The in vitro cholesterol removal rates of live and heated to dead cells of the positive control probiotic Lactobacillus plantarum ATCC 14917 were (29.77±1.7%) and (19.95±8.84%, respectively). The in vitro cholesterol-lowering rate of bacteriophage DC4 was higher than that of the positive control Lactobacillus plantarum ATCC14917.According to the results of the statistical analysis it can be tentatively concluded that Lactobacillus plantarum DC4 has a good in vitro cholesterol-lowering effect and the in vitro cholesterol removal rate was higher than that of the positive control strain. From the results of the experimental analysis, it can be concluded that the results of cholesterol removal rate between strains indicate that they are significantly different from each other ($p<0.05$) [11].

Note: Positive strains are Lactobacillus plantarum ATCC 14917. DC4 is Lactobacillus plantarum DC4.All data from the experiments were labeled as mean ± standard deviation (±s) and compared between groups using SPSS 23 software. One-way ANOVA was then used. Data within each group were analyzed using a paired t-test $p<0.05$ difference is significant. Different letters represent significant differences between the two [12].

3.2. Determination of Cholesterol Adsorption by Lactobacillus Plantarum DC4

Cholesterol adsorption by live and dead cells of Lactobacillus plantarum DC4 was observed by electron microscopy. According to the results shown in Figure 2, positive strains are positive control ATCC19417 and DC4 is Lactobacillus plantarum DC4.The adsorption rate of dead Lactobacillus plantarum DC4 on cholesterol was found to be about 30 %. In contrast, the cholesterol adsorption rate of live Lactobacillus plantarum DC4 was about 72 % [13]. The results indicate that live Lactobacillus plantarum DC4 has good cholesterol adsorption capacity. Both its live and dead cell organisms adsorbed cholesterol, but the live cells had a stronger adsorption capacity for cholesterol aggregation.

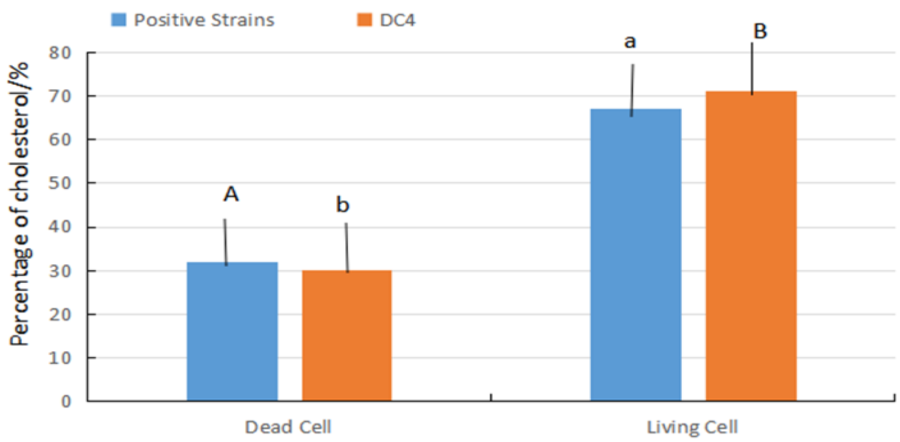

Figure 2: Electron microscopic results of cholesterol

Note: Positive Strains is the positive control ATCC14917 and DC4 is Lactobacillus plantarum DC4.The experimental data were all labeled according to the mean ± standard deviation (±s). And SPSS 23 software was used for comparison between groups. One-way ANOVA was used. Data within groups were analyzed by paired t-test. The $p<0.05$ difference is significant. Different letters represent significant differences between the two.

3.3. Effect of Lactobacillus Plantarum DC4 on Serum Cholesterol in Mice

The use of high-fat diet induction can cause a significant trend of increasing the concentration of serum cholesterol in rats. The experimental animals were randomly divided into three groups, the first group was the normal control group, fed with normal chow. The second group was a high-fat model group, used to establish a high-fat rat model, fed a high-fat diet. The third group was divided into experimental groups and fed with both high-fat chow and experimental strain (Lactobacillus plantarum DC4). After eight weeks of feeding in the above manner, the cholesterol levels in the serum of mice were measured and plotted in Figure 3 [14]. The experimental results showed that the cholesterol level in the serum of mice in the normal group was 1.75 mM. Serum cholesterol levels were highest in mice fed high-fat diets, with mice having a serum cholesterol level of approximately 2.85 mM. Mice fed a mixture of high-fat chow and Lactobacillus plantarum DC4 had low serum cholesterol levels compared to those in the high-fat group. It was about 2.25 mM. It was tentatively concluded that the cholesterol concentration in the serum of mice decreased significantly after Lactobacillus plantarum DC4 intervention.

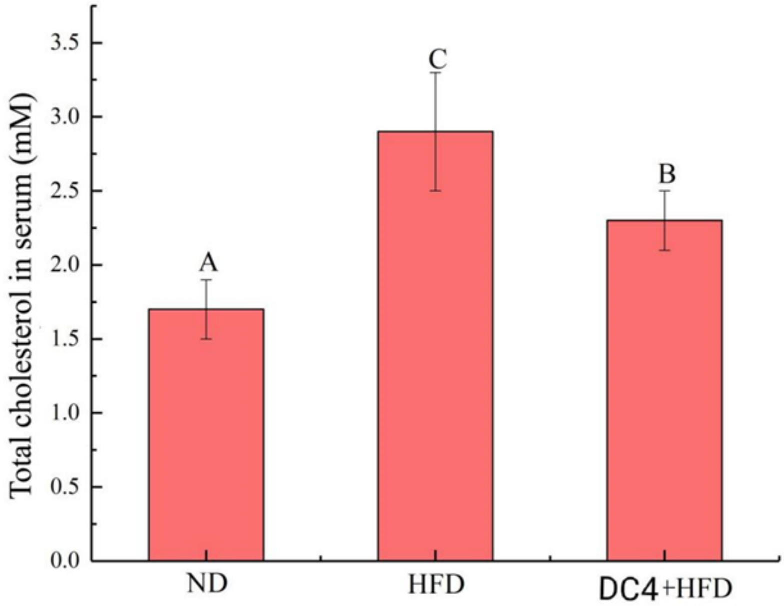

Figure 3: Effect of Lactobacillus plantarum DC4 on cholesterol in mice serum

Note: Blank control (ND) group: fed normal chow; high-fat (HFD) group: fed high-fat chow. Lactobacillus plantarum DC4 (HFD + DC4) mixed group: fed high-fat diet and Lactobacillus plantarum DC4 mixed group, all data of the experiments were labeled as mean ± standard deviation (±s) and compared between groups using SPSS 23 software. Then the data were analyzed according to one-way ANOVA. Data within each group were analyzed by paired t-test with $p<0.05$ significant difference, and different letters represent significant differences between them [15].

3.4. Effect of Lactobacillus Plantarum DC4 on Bile Acid Metabolism in Hyperlipidemic Mice

Determination of bile acid content in fecal samples of mice and drawing of bile acid spectrum in fecal samples of mice [16]. The feces of each mouse was sampled in batches of three repetitions, and the changes in bile acid metabolism were detected. The mice were divided into three groups, and the feces of Lactobacillus plantarum DC4 were extracted before, during and after the intervention on the mice with hyperlipidemia, and the levels of bile acid metabolism were detected and plotted as shown in Figure. 4 [17]. The levels of bile acid in the feces of the mice in the three groups before the intervention were about 0.85 g, 0.9 g and 0.86 g, respectively. The fecal bile acid content of mice in the three groups in the intervention was approximately 0.95 g, 1.02 g and 1.00 g, respectively. The fecal bile acid content of mice in the three groups after the intervention was about 1.2 g, 1.32 g and 1.24 g, respectively. The bile acid metabolism levels of the three groups of mice were significantly different, and the highest bile acid metabolism levels were found in the feces of mice after Lactobacillus plantarum DC4 intervention. And the bile acid levels of the three groups of mice showed an increasing trend before, during and after Lactobacillus plantarum DC4 intervention, indicating that this strain can have an effective promotion of bile acid metabolism in mice with hyperlipidemia.

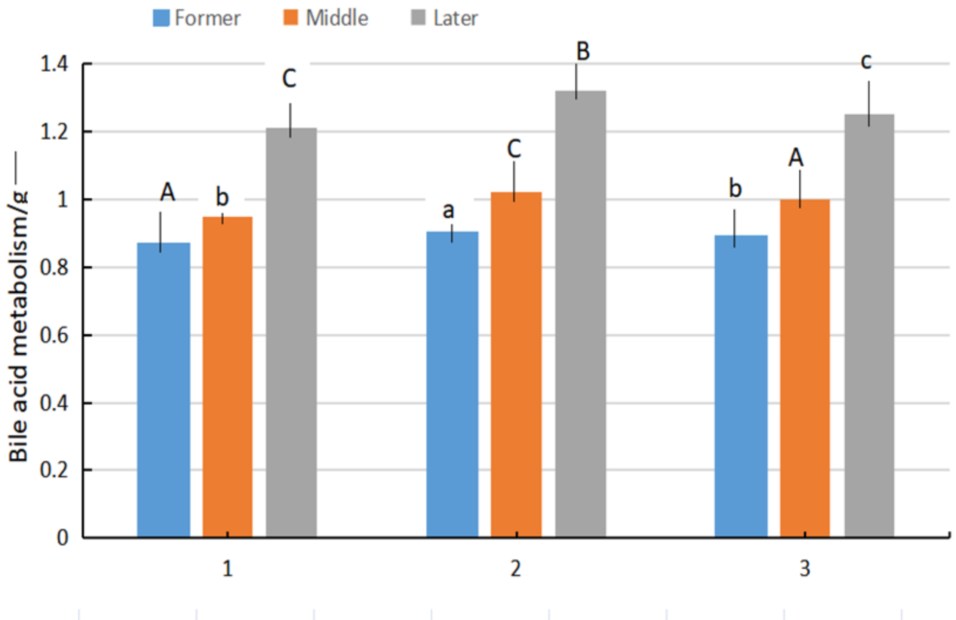

Figure 4: Effect of Lactobacillus plantarum DC4 on bile acid metabolism in hyperlipidemic mice

Note: 1, 2, 3 denote three groups with equal number of hyperlipidemic mice. Former, Middle, and Later indicate the levels of bile acid metabolism in mouse feces before and after Lactobacillus plantarum DC4 intervention, respectively. The experimental data were all labeled according to the mean ± standard deviation (±s) and compared between groups using SPSS 23 software, and then analyzed according to one-way ANOVA. Data within each group were analyzed by paired t-test with a significant difference of $p<0.05$, with different letters representing significant differences between the two.

4. Experimental Conclusion and Discussion

In this experiment, we found that Lactobacillus plantarum DC4 had a higher in vitro cholesterol-lowering rate than the positive control Lactobacillus plantarum ATCC14917 after 24 h of culture, and had a stronger in vitro cholesterol-lowering ability. And both live and dead cells of Lactobacillus plantarum DC4 have certain adsorption capacity for cholesterol, as shown in Figure 3, B and C. B indicates the adsorption aggregation of dead Lactobacillus plantarum DC4 cells for cholesterol, while C indicates the adsorption of live Lactobacillus plantarum DC4 with cholesterol, and the adsorption aggregation capacity of live cells is stronger compared with dead Lactobacillus plantarum DC4 cells. Lactobacillus plantarum DC4 could also effectively reduce cholesterol levels in hyperlipidemic mice through a pathway that promotes bile acid metabolism in hyperlipidemic mice, and thus.

In conclusion, Lactobacillus plantarum DC4 has excellent properties and can be used as a potential strain for the development of cholesterol-lowering functional foods, with promising applications.

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