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Screening and characterization of lactic acid bacterial strains that produce fermented milk and reduce cholesterol levels

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ABSTRACT

Objective: To screen for and characterize lactic acid bacteria (LAB) strains with the ability to produce fermented milk and reduce cholesterol levels.
Methods: The strains were isolated from traditional fermented milk in China. *In vitro* and *in vivo* evaluation of cholesterol-reduction were used to identify and verify strains of interest. Characteristics were analyzed using spectrophotometry and plate counting assays.
Results: The isolate HLX37 consistently produced fermented milk with strong cholesterol-reducing properties was identified as *Lactobacillus plantarum* (accession number: KR105940) and was thus selected for further study. The cholesterol reduction by strain HLX37 was 45.84%. The isolates were acid-tolerant at pH 2.5 and bile-tolerant at 0.5% (w/v) in simulated gastric juice (pH 2.5) for 2 h and in simulated intestinal fluid (pH 8.0) for 3 h. The auto-aggregation rate increased to 87.74% after 24 h, while the co-aggregation with *Escherichia coli* DH5 was 27.76%. Strain HLX37 was intrinsically resistant to antibiotics such as penicillin, tobramycin, kanamycin, streptomycin, vancomycin and amikacin. Compared with rats in the model hyperlipidemia group, the total cholesterol (TC) content in the serum and the liver as well as the atherogenic index (AI) of rats in the viable fermented milk group significantly decreased by 23.33%, 32.37% and 40.23%, respectively. Fewer fat vacuoles and other lesions in liver tissue were present in both the inactivated and viable fermented milk groups compared to the model group.
Conclusion: These studies indicate that strain HLX37 of *L. plantarum* demonstrates probiotic potential, potential for use as a candidate for commercial use for promoting health.

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Introduction

Cholesterol is an indispensable nutrient in the human body,[1] as it serves as a component of the cell membrane and as a precursor for cholic acid and steroid hormone synthesis. However, excess cholesterol is a major contributor to hyperlipidemia and cardiovascular and cerebrovascular diseases.[2,3] According to the statistics from the National Health and Family Planning Commission of China (2015), cardiovascular and cerebrovascular diseases have become a leading cause of death worldwide, with as many as 15 million people dying from these diseases annually. Research shows that the probability of suffering from cardiovascular disease decreases by 2–3%[4] with every 1% decrease in the serum cholesterol level. Therefore, an effective method for reducing high cholesterol to a healthy level could protect against these diseases.

Studies have shown that some therapeutics, such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, effectively reduce serum cholesterol.[5] Along with these therapeutics, a person's diet should also be regulated to include foods effective at preventing and reducing cholesterol. Numerous experiments have demonstrated that probiotic lactic acid bacteria can also reduce cholesterol *via* adsorption, conversion, co-precipitation and bile salt hydrolase (BSH) enzymatic degradation.[5,6] However, the oral intake of probiotics is not palliative for most people.

Fermented milk obtained through the lactobacillus fermentation of fresh milk is a dairy product with a number of nutritional and health advantages. The fermentation process also involves the growth of lactic acid bacteria that convert the lactose and milk protein into lactic acid, galactose, amino acids and small peptides, which are easily digested and absorbed by the human body. Moreover, the nutritional value of fermented milk products generated by various lactic acid bacteria can also be different. In addition to satisfying nutritional requirements, some fermented milk products can improve the absorption and uptake of nutritive substances, such as calcium-containing substances. There are numerous lactic acid bacteria that can inhabit the internal wall of the intestinal tract and form microbial communities and a biological barrier. Inhibition of the growth of pathogenic bacteria in the intestinal tract[7,8] and the regulation of the balance of intestinal microflora in such a way may have effects similar to those of probiotics and metabolites.[9,10]

For these reasons, we set out to screen for lactic acid bacterial (LAB) strains capable of producing fermented milk and reducing cholesterol levels for use as starter cultures. Furthermore, this study investigated the ability of these strains in fermented milk to reduce cholesterol in high-fat rats, and evaluated these strains as candidate commercial probiotics to promote health.

Materials and methods

Samples and media

Traditional fermented milk samples were screened for lactic acid bacterias trains able to reduce cholesterol levels. Selected strains were cultivated in fresh milk collected from a farmer in Mangdang Town, Yanping District, Nanping City, in the Fujian Province of China (26.510°N, 118.090°E). Traditional fermented milk was made as follows: the fresh milk from Chinese Holstein cows was incubated at 15 °C for 35 days to ferment; when the pH value reached approximately 4.4, fermented milk had been produced. Man, Rogosa and Sharpe (MRS) medium was used for culturing the LAB. A basal diet was obtained from the Center for Disease Control and Prevention (Fujian, China) and contained 10% moisture, 22% crude protein, 4% crude fat, 5% crude fiber, and 8% crude ash. A hypercholesterol forage consisted of 57% basal diet, 13% lard, 5% peanuts, 2% sesame oil, 20% sucrose, 3% cholesterol (Zhengzhou Tianjian Food Technology Co., Ltd, Hunan Province, China), and 0.1% sodium cholate (Zhengzhou Tianjian Food Technology Co., Ltd., Hunan Province, China).

Animals

Sprague-Dawley (SD) male rats (weighing 150–170 g) were purchased from the Hayes Lake Laboratory Animal Co., Ltd. (Shanghai, China) [license number: SCXK (Shanghai 2007-0005)]. The rats were housed under standard controlled laboratory conditions (temperature of 24 ± 3 °C, humidity of 50 ± 10% and 12 h light/12 h dark cycle), with unrestricted access to food and water. All animals were handled in accordance with the animal care and use guidelines established by the Fujian University of Traditional Chinese Medicine (Fujian, China), which entailed minimizing both the number and suffering of the animals used for the experiments. All experimental procedures involving rats were approved by the Fujian Association for Laboratory Animal Science (FALAS).

Screening for strains capable of fermenting milk and reducing cholesterol levels

Natural fermented milk samples (1 mL) were serially diluted, and 10^{-5}, 10^{-6} and 10^{-7} dilutions in saline were plated on solid MRS agar plates. The plates were incubated at 42 °C for 2 days. Colonies with phenotypes and characteristics similar to those of *Lactobacillus* were selected and sub-inoculated into MRS medium three times. The isolated strains were inoculated into 10 mL of MRS medium and incubated anaerobically at 42 °C for 1 day. A 1.0-mL aliquot of the culture broth was sub-inoculated into a fermenter containing 100 mL of pasteurized milk (Changfu Dairy Group Co., Ltd. Fujian Province, China), which had been sterilized at 115 °C for 20 min and incubated at 42 °C for 12 h. Following fermentation, any strain that consistently resulted in solidified fermented milk with a pleasing smell and a pH value ≤4.4 was selected, labeled and stored in 20% glycerol at −20 °C until further analysis. To screen for strains that could reduce milk cholesterol levels, a 1-mL aliquot of the culture broth from the tested fermentation strains was transferred into a fermenter containing 100 mL of cholesterol (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) screening medium and incubated at 42 °C for 2 days. Any strain that produced turbidity in the culture broth was tested for cholesterol reduction ability. LAB cultures were grown in MRS medium supplemented with water-soluble cholesterol (approximately 200 mg/L) for

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