Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.201414B54

Effects of lactic acid bacteria isolated from fermented mustard on lowering cholesterol

Shu Chen Wang[1], Chen Kai Chang[1], Shu Chang Chan[2], Jiunn Shiuh Shieh[1], Chih Kwang Chiu[1], Pin-Der Duh[1*]

[1]*Department of Food Science and Technology, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Section 1, Pao-An, Jen-Te District, Tainan, Taiwan*

[2]*Department of Biotechnology, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Section 1, Pao-An, Jen-Te District, Tainan, Taiwan*

PEER REVIEW

Peer reviewer

Hau-Yang Tsen, Professor, Department of Food Nutrition, Hung-Kuang University, No. 34, Chung-Chi Rd, Shalu, Taichung County 433, Taiwan. Tel: +886422870162, Fax: +886 422870162, E-mail: hytsen@sunrise.hk.edu.tw

Co-reviewers: Ekachai Chukeatirote, Thailand. S. Garcha, Patiala, India. Shinobu Watanabe, Japan. Tohru Gonoi, Chiba, Japan.

Comments

This work shows strain B0006, B0007 and B0022 with remarkable cholesterol-lowering activity are acid and bile salt tolerance. In particular, strain B0007 and B0022 displayed strong adherence to Caco-2 cells. Considering the adherent activity, we suggest that strains B0007 and B0022 may be potential alternatives for supplement to lower serum cholesterol.

Details on Page 527

ABSTRACT

Objective: To evaluate the ability of lactic acid bacteria (LAB) strains isolated from fermented mustard to lower the cholesterol *in vitro*.

Methods: The ability of 50 LAB strains isolated from fermented mustard on lowering cholesterol *in vitro* was determined by modified o-phtshalaldehyde method. The LAB isolates were analyzed for their resistance to acid and bile salt. Strains with lowering cholesterol activity, were determined adherence to Caco-2 cells.

Results: Strain B0007, B0006 and B0022 assimilated more cholesterol than BCRC10474 and BCRC 17010. The isolated strains showed tolerance to pH 3.0 for 3 h despite variations in the degree of viability and bile-tolerant strains, with more than 10^8 CFU/mL after incubation for 24 h at 1% oxigall in MRS. In addition, strain B0007 and B0022 identified as *Lactobacillus plantarum* with 16S rDNA sequences were able to adhere to the Caco-2 cell lines.

Conclusions: These strains B0007 and B0022 may be potential functional sources for cholesterol-lowering activities as well as adhering to Caco-2 cell lines.

KEYWORDS

Cholesterol-lowering activity, Probiotic, Lactic acid bacteria, Acid, Bile tolerance

1. Introduction

Overmuch cholesterol in the blood and diet is a major risk factor for coronary heart disease and colon cancer[1]. For each 1 mmol above the normal cholesterol level, the risk of coronary heart disease was approximately 35% higher and coronary death was 45% higher[2]. Recently, lactic acid bacteria (LAB) have attracted attention as potential cholesterol-lowering agent[3]. Consumption of dairy products containing probiotics has been proposed as a means to lower serum cholesterol[4]. Several studies indicated consumption of certain cultured dairy products could lower total plasma cholesterol and low-density lipoprotein cholesterol[1,5]. The reduction of serum cholesterol could be an important health

*Corresponding author: Pin-Der Duh, Professor, PhD, Department of Food Science and Technology, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Section 1, Pao-An, Jen-Te District, Tainan, Taiwan.

E-mail: ipdduh@mail.chna.edu.tw

Tel: 88662660611

Fax: 88663663756

Foundation Project: Supported by research grants from the Ministry of Economic Affairs of Taiwan, the Republic of China with grant NO. 98-EC-17-A-17-S1-132.

Article history:

Received 19 Apr 2014

Received in revised form 8 May, 2nd revised form 18 May, 3rd revised form 23 May 2014

Accepted 13 Jun 2014

Available online 28 Jul 2014

benefit of LAB, demonstrated in human, mouse, pig studies and rats[5-7]. Therefore, the investigation of effective natural ingredients from food that could decrease concentrations of serum cholesterol has recently drawn a great deal of attention[3]. Many kinds of *Lactobacillus* cultures exerted potential hypocholesterolemic activity[5,8].

Fermented mustard (picked mustard green) is made from green mustard and its production is a spontaneous fermentation process by a mixed microbial population mainly composed of LAB. The aim of this study was to screen lactobacilli with probiotic characteristics isolated from traditionally fermented mustard, and to determine the effect of the screened *Lactobacillus* strains for their ability to lower cholesterol and the strains were also identified.

2. Materials and methods

2.1. Traditional Taiwan fermented mustard samples

The liquor samples of fermented mustard were collected from the farms of central and southern Taiwan.

2.2. Isolation of LAB

The diluted liquor samples were spread on the surface of MRS agar containing 5 g/L calcium carbonate and then incubated at 37 °C for 1-2 d. Colonies of clear zones on MRS agar plates were randomly selected and purified. Only Gram-positive and catalase-negative strains were taken as presumptive LAB and stored at 4 °C in MRS agar plate.

2.3. Cholesterol removal

The cholesterol removal was performed using procedures described by Wang *et al*[1]. In brief, freshly prepared MRS broth was supplemented with 0.30% oxigall (Sigma, MO, USA) as a bile salt. Water-soluble cholesterol (polyoxyethylene cholesteryl sebacate) was filter-sterilized and added to the broth at a final concentration of 200 to 300 μg/mL, inoculated with each strain and incubated anaerobically at 37 °C for 20 h. After the incubation period, cells were centrifuged and the remaining cholesterol concentration in the broth was determined using a modified colorimetric method as described by Wang *et al*[1]. The aliquot (100 μL) were added with 100 μL of KOH (33% w/v) and 200 μL of absolute ethanol, vortexed for 1 min, and heated at 37 °C for 15 min. After cooling, 200 μL of distilled water and 300 μL of hexane were added and vortexed for 1 min. The hexane layer (100 μL) was transferred into a glass tube and evaporated under nitrogen. The residue was immediately dissolved in 200 μL of o-phthalaldehyde reagent. After complete mixing, 50 μL of concentrated sulfuric acid were added and the mixture was vortexed for 1 min. Absorbance was read at 540 nm with ELISA reader (Multiskan EX, Labsystem, Gyeongbuk, Korea) after 10 min. All experiments were replicated twice.

2.4. Cholesterol removal by dead and resting cells

Freshly prepared MRS broth containing 0.30% oxigall was inoculated with each strain of LAB and incubated at 37 °C for 20 h. Cells were harvested after the incubation period by centrifuging at 10000 r/min (Microspin 24, Sorvall Instruments, Melbourne, Australia) at 4 °C for 10 min. The cell pellet was washed twice with sterile distilled water. For preparation of heat-killed cells, the cell pellet was suspended in 10 mL of sterile distilled water and autoclaved for 15 min at 121 °C. For preparation of resting cells, the cell pellet was suspended in 10 mL of 0.05 mol/L sterile phosphate buffer (pH 6.8)[9]. All samples were suspended in MRS broth containing 0.30% oxigall and water-soluble cholesterol and assayed for cholesterol content as mentioned above. The experiments were repeated twice.

2.5. Acid tolerance

Acid tolerance of the cultures was investigated by incubating the organisms in MRS broth supplemented with 0.30% oxigall. The pH was adjusted to 3.0 and 2.0 with HCl and cultures were incubated at 37 °C for 3 h. Each of the isolated LAB was subcultured at least 3 times before experimental use, followed by centrifugation after the final subculture, inoculation (10% v/v) into the broth, and growth monitoring using the plate count method[9]. The experiments were repeated twice.

2.6. Bile tolerance

The LAB isolates were analyzed for their resistance to bile salt. The MRS broths at concentrations of 0%, 0.5% and 1.0% (w/v) of oxigall were prepared and dispensed in 10 mL volumes and sterilized by heating 121 °C for 15 min. Each of the isolated LAB was subcultured at least 3 times before experimental use, followed by centrifugation after the final subculture, inoculation (10% v/v) into the broth, and growth monitoring using the plate count method[9]. The reaction mixture and MRS broth were incubated at 37 °C for 24 h. All the experiments were repeated twice.

2.7. Adhesion assay

The Caco-2 cell-lines were purchased from the Bioresources Collection and Research Center (BCRC), Hsin-Chu, Taiwan. Cells were grown routinely in Dulbeco's modified Eagle's minimal essential medium (DMEM; GIBCO BRL Laboratories, NY, USA) containing 1.0 mmol/L sodium