HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Effect of lactic acid bacteria isolated from fermented mustard on immunopotentiating activity

Chen-Kai Chang[1], Shu-Chen Wang[1], Chih-Kwang Chiu[1], Shih-Ying Chen[2], Zong-Tsi Chen[3], 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 Health and Nutrition, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Section 1, Pao-An, Jen-Te District, Tainan, Taiwan*

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

PEER REVIEW

Peer reviewer

Luís Rodrigues da Silva, PhD, REQUIMTE, Porto University, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal.
Tel: +351914662884
E-mail: luisfarmacognosia@gmail.com

Comments

The present manuscript is well written, with good methodologies and the results are well discussed. The results herein obtained are very interesting and reveal a good potential for supplement to induce immunopotentiating activities.
Details on Page 285

ABSTRACT

Objective: To investigate the effect of lactic acid bacteria isolated from fermented mustard on immunopotentiating activity

Methods: One hundred and fifty nine strains of lactic acid bacteria isolated from traditional Taiwan fermented mustard were evaluated for their immunopotentiating activity on a murine macrophage cell line RAW 264.7.

Results: Of the strains, pronounced increases in the levels of nitric oxide (NO), tumor necrosis factor-α and interleukin-6 were observed in strains B0040, B0110 and B0145. Among them, strain B0145 had the highest NO and tumor necrosis factor-α generation in RAW 264.7 cells; strains B0040 and B0110 were also superior to that of *Lactobacillus casei*. These results demonstrated that NO and cytokines were effectively induced when the bacterial stimulants were treated with macrophages. In addition, strains B0040 and B0110 were identified as *Lactobacillus plantarum*, and B0145 as *Weissella cibaria* using 16S rDNA analysis.

Conclusions: The results implicated selected strains may be regarded as a biological response modifier and had a broad application prospects in exploiting new functional food or as a feed additive.

KEYWORDS
Immunopotentiating activity, Interleukin-6, Lactic acid bacteria, Nitric oxide, Tumor necrosis factor-α

1. Introduction

Some lactic acid bacteria (LAB) are believed to play important roles in the development and maintenance of health benefit of host. These possible health effects include assimilation of cholesterol[1], reducing dental caries[2], modulating the immune system, increasing the antibacterial, anticancer and antimutagenic activities and preventing cancer recurrence[3-5]. Recently, a considerable attention has been focused on immunological functions of LAB as a promising strategy for health-promoting

*Corresponding author: Pin-Der Duh, 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.
Tel: 88662660611
Fax: 88663663756
E-mail: ipdduh@mail.cnu.edu.tw

Foundation Project: This research work was supported by research grants from the Ministry of Economic Affairs of the Republic of China (101-EC-17-A-18-S1-228).

Article history:
Received 30 Dec 2014
Received in revised form 11 Jan 2015
Accepted 2 Feb 2015
Available online 12 Mar 2015

effects. For instance, some LAB stimulate natural killer cell and modulate systemic inflammation, which contribute to exhibit antitumor and antiinfectious activity[6-8]. In addition, activated macrophages are able to recognize and lyse tumor cells which are resistant to cytostatic drugs and can play a key role in novel immunotherapeutic approaches to the treatment of cancer[9]. Previous studies noted that activated macrophages were able to induce the production of hydrogen peroxide, nitric oxide (NO), and cytokines, such as interferon-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-6 which conducted a pivotal function in a variety of immune response[10-12]. In other words, the intake of LAB which activated macrophages may enhance resistance against infection by pathogenic organism and help in the prevention of cancer[4,5].

In Asia, fermented fruits and vegetables products had a long history in human nutrition from ancient ages and were associated with the several social aspects of different communities. Recent studies were conducted to evaluate traditional fermented vegetables as potential natural sources of probiotic bacteria[13]. Suan-tsai is traditional fermented mustard which is widely used in Taiwan. It is made from green mustard and its production is a spontaneous fermentation process by a mixed microbial population mainly composed of LAB[14]. However, the information related to immunological functions of LAB isolated from traditional Taiwan fermented mustard is limited. The aim of this study was to evaluate the *in vitro* effect of LAB isolated from suan-tsai on the induction of NO and cytokines such as TNF-α and IL-6 in RAW 264.7 macrophage cells and the strains were also identified.

2. Materials and methods

2.1. Microorganism and culture condition

One hundred and fifty nine strains of LAB isolated from suan-tsai were used in the experiments. In addition, the probiotic lactobacilli *Lactobacillus casei* (*L. casei*), isolated from a commercial yogurt of Yakult Co., Ltd. (Taipei, Taiwan), was also included in the study. All strains were maintained at -80 ˚C in 20% (v/v) glycerol. Prior to each of the experiments, all LAB were cultured in de Man, Rogosa and Sharpe broth (MRS broth; Difco Laboratories, Detroit, MI, USA) at 37 ˚C for 18 h two or three times.

2.2. Preparation of bacterial stimulants

After cultivation, the bacteria were collected by centrifugation and washed twice with phosphate-buffered saline (pH 7.2). The spent culture supernatant (SCS) of LAB was collected by centrifugation at 8 500 rcf for 10 min at 4 ˚C and filtered through a 0.22 μm pore filter unit (Millipore, Bedford, MA). For all experiments involving heat-inactivated bacterial preparations, samples of freshly-prepared cultures were enumerated using the appropriate agar, while additional samples of the same cultures were heat-inactivated (95 ˚C/30 min). Successful heat-killing was confirmed by the absence of bacterial growth on MRS agar plates. Subsequently, the concentration of the heat-inactivated preparation was adjusted in lieu of the live plate counts and used for further experimentation. For the treatment of RAW 264.7 cells, the viable and heat-inactivated bacteria were then suspended in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St. Louis, USA) at 10^9 CFU/mL, respectively. The bacterial preparations were stored at -80 ˚C until use.

2.3. Cell culture

The mouse macrophage cell line RAW 264.7 was purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan) and maintained in DMEM complete medium, supplemented with 10% fetal bovine serum, streptomycin (100 μg/mL), and penicillin (100 IU/mL) at 37 ˚C in a 5% CO_2 humidified incubator.

2.4. Determination of NO and cytokines

For the experiments, RAW 264.7 cells were cultured in 24-well tissue culture plates with a density of 5×10^5 cells/mL. To the wells, either viable or heat-inactivated bacteria at representing a bacteria:cell ratio of 25:1 were added. After 24 h incubation (37 ˚C, 5% CO_2), the supernatant were collected and analyzed for NO and cytokines.

The cultured supernatant was collected and analysed for NO production via the Griess reaction[10]. Briefly, the supernatant was mixed with an equal volume of Griess reagent (1% naphthylethylenediamine dihydrochloride and 1% sulfanilamide in 5% phosphoric acid) in 96-well plates. After the mixed solution had reacted for 10 min at room temperature and absorbance was measured at 540 nm, NO concentrations were calculated on the basis of a standard curve prepared using sodium nitrite.

The concentrations of IL-6 and TNF-α were assayed with commercial ELISA kits (Pharmingen, CA, USA) according to the manufacturer's recommendations, and absorbance was measured at 450 nm using the 96-well plate reader.

2.5. Cell viability

The cytotoxicity of the LAB on RAW 264.7 cells was estimated based on the method of 3-(4,5-dimethylthiazol-2-yl)-2,5-