The Influence of Fermentation by Different Lactobacillus on the Free Radical Scavenging Activity of Burdock and Variations of Its Active Components

Chi-Ting Horng[1,2], Shih-Chiang Lee[3], Rei-Chu Chang[4], Wan-Ping Lee [5], Feng-Lang Lin[2], Chin-Wen Hsu[6], Fu-An Chen[2,*]

[1] Department of Ophthalmology, Kaohsiung Armed Forces General Hospital, Kaohsiung, Taiwan, ROC
[2] Department of Pharmacy & Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung, Taiwan, ROC.
[3] Dong Yuan Biotech Pharmaceutical Co. Ltd., Kaohsiung, Taiwan.
[4] Department of Food Science and Technology, Tajen University, Pingtung, Taiwan, ROC
[5] Department of Nursing, Tajen University, Pingtung, Taiwan, ROC
[6] Department of Surgery, Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan, ROC
h56041@gmail.com

Abstract: Burdock (*Arctium lappa* L.) is a nutritious plant which is commonly cultivated in Taiwan and Japan. The purpose of this study is to explore the effect of fermentation by different lactobacillus on the free radical scavenging activity of burdock and variations of its active components. Four lactobacillus as *Lactobacillus casei subsp. casei* (Orla-Jensen) Hansen and Lessel (BCRC No.10697), *Lactobacillus delbrueckii subsp. bulgaricus* (Orla-Jensen) Weiss et al (BCRC No.10696), *Lactobacillus plantarum subsp. plantarum* (Orla-Jensen) Bergey et al (BCRC No.10069) and *Streptococcus thermophilus* (Orla-Jensen) (BCRC No.14086) were used to ferment burdock for 48 hours. The amount of lactic acid bacteria (LBA), sweetness, pH, total polyphenols and the free radical scavenging activity, using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay were measured. The result showed *Lactobacillus casei subsp. casei* (Orla-Jensen) Hansen and Lessel (BCRC No.10697) had better effect on LBA populations, total polyphenols and free radical scavenging activities compared with other three lactobacillus. This result provides important information on developing fermented burdock antioxidant dietary supplements.

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1. Introduction

Burdock (*Arctium lappa* L.) has long been cultivated as a vegetable in Taiwan for dietary use[1]. Burdock is also used as a folk medicine as a diuretic and antipyretic[2]. It has become a popular health drink in Taiwan in the last decade. Several studies have reported that the root of burdock possesses various pharmaceutical activities including antibacterial activity[3,4], desmutagenic activity[5], antioxidant ability[6-9], hepatoprotective effect[10,11], gastroprotective activity[12,13], hypoglycemic activity [14,15], hypolipidemic activity [16], sexual behavior enhancement[15] and anti-inflammatory activity[16], among which the gastroprotective activity, hepatoprotective efficacy, anti-inflammatory activity[7], and antioxidant activity are associated with the free radical scavenging activity[8].

Fermentation using yeast or lactic acid bacteria has long been applied in food industry due to its beneficial effects in flavor development, in inhibition of spoilage bacteria and pathogens, in intestinal health and other health benefits related to cancer prevention, blood cholesterols levels and immune competence, which could be resulted from the modification and/or creation of nutrient, botanically-active components and microbial metabolites [18-20]. The present study is thus to examine the influence of fermentation by different lactobacillus on the free radical scavenging activity of burdock and variations of its active components.

2. Material and Methods

Material

Burdock (*Arctium lappa* L.) was obtained from the Gueilai Community Developmental Institute in Pingtung County, southern Taiwan. Lactobacillus： *Lactobacillus casei subsp. casei* (Orla-Jensen) Hansen and Lessel (BCRC No.10697), *Lactobacillus delbrueckii subsp. bulgaricus* (Orla-Jensen) Weiss et al (BCRC No.10696), *Lactobacillus plantarum subsp. plantarum* (Orla-Jensen) Bergey et al (BCRC No.10069) and *Streptococcus thermophilus* (Orla-Jensen) (BCRC No.14086) were purchased from the

Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan. De Man, Rogosa, Sharpe (MRS) broth was the product of Difco (Becton, Dickinson and Company, USA). Methanol and acetone were the product of Tedia and Mallinckrodt (USA), repectively. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma (USA). Folin-ciocalteu reagent was purchased from Merck (Germany). All other chemicals were of analytical reagent grade.

Burdock fermentation

Six hundred grams of the root of burdock was extracted by 3 L of hot distilled water for 30 min at 100 °C. The aqueous burdock extract solution was filtered through filter paper and a filter funnel. After cooling, 100 mL of aqueous burdock extract solution was placed into a bottles and further sterilization in an autoclave. four lactobacillus strains as mentioned previously were inoculated for fermentation at 37 °C. The physicochemical property, total polyphenols content and free radical scavenging activity of burdock ferment liquid (BFL) were determined after fermentation for 48 hrs.

Physicochemical properties of BFL

Lactic acid bacteria (LBA) populations were counted using standard methods according to CNS 10890. Ten fold dilutions beginning with 1 ml of a sample were added to 9 ml of normal saline solution (0.85% NaCl) to obtain a 10^{-1} dilution. Appropriate dilutions were used for the pour plate counting of LAB. The medium used was de Man Rogosa Shape (MRS) for incubation for 48 h at 37±1℃ and then counting LAB in term of CUF/mL. The pH of BFL was measured by means of a Mettler Toledo Delta 320 pH meter (Mettler-Toledo, Greifensee, Switzerland). Sugars content of BFL were determined using an Atago digital hand-held refractometer (Tokyo, Japan) in terms of ºBrix. Three replicates were used for BFL sample.

Determination of total polyphenols in BFL

Total polyphenols in BFL were measured spectrophotometrically using the Folin–Ciocalteu reagent based on a colorimetric oxidation/reduction reaction[13,21]. 1 mL of Folin–Ciocalteu reagent (diluted 10 times with water) was added to 0.2 mL of diluted aqueous acetone sample. After that, 0.8 ml of 7.5 % Na_2CO_3 was added and mixed thoroughly. The absorbance was measured at 765 nm (Hitachi, Tokyo, Japan) after 0.5 h of standing. The amount of total polyphenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions and expressed as mg gallic acid /g BFL. All measurements were done in triplicate.

DPPH free radical scavenging activity of BFL

The free radical scavenging activity of BFL was evaluated using DPPH free radical-scavenging assay as described previously[22]. A stock solution (1 mg/mL) of each extract was prepared and diluted with methanol into various concentrations. An aliquot of 50 μL of each dilution was transferred into a 96-well microplate (NUNC, Roskilde, Denmark). A working solution of DPPH (250 μM) in methanol was freshly prepared and then an aliquot of 150 μL was added to each well. The DPPH scavenging percentage was measured at 490 nm on an ELISA reader (ThermoLabsystems, Cheshire, UK after incubation for 0.5 h. Each dilution was performed at least in triplicate.

3. Results and discussion

In the present study, four lactobacillus strains as *Lactobacillus casei subsp. casei* (Orla-Jensen) Hansen and Lessel (BCRC No.10697), *Lactobacillus delbrueckii subsp. bulgaricus* (Orla-Jensen) Weiss et al (BCRC No.10696), *Lactobacillus plantarum subsp. plantarum* (Orla-Jensen) Bergey et al (BCRC No.10069) and *Streptococcus thermophilus* (Orla-Jensen) (BCRC No.14086) were used to ferment burdock and the effects of fermentation by different LBA on the functional ingredients and free radical scavenging activity were measured in order to find the appropriate fermentation conditions of lactobacillus. The general physicochemical properties after each of the total viable LBA count and the burdock fluid had been inoculated with the four lactobacillus. After fermentation, the amount of total viable bacterial in burdock ferment liquid (BFL) was measured and the result was 10697 ＞ 10069 ＞ 10696≒14086 (Fig. 1). For a probiotic product to be beneficial, it must contain at least 10^6 CFU/mL of viable LBA according to the criteria of probiotic products in Taiwan.

Fig. 1 shows the LBA in the four lactobacillus strains after 48 hours fermentation were higher than 10^6 CFU/mL, and the colony 10697 has the largest amount of lactic acid bacteria compared to other lactobacillus strains. These results can be applied to the development of probiotic products. The pH among lactobacillus strains were between 2-5 which is similar to juice and carbonated drinks and the result was 14086＞10696＞10697＞10069 (Fig. 2). On the sweet determination standard, higher than 15 degree is defined as highly sweetened; between 12-15 degree is defined as very sweet; between 10-12degree as defined as slightly sweet; between 8-10 degree as

defined as a bit sweet; and less than 8 degree is defined as non sweetness. There was no significantly different in measuring of sweetness among the four lactobacillus strains and the results of the four stains were all under degree 8 (Fig. 3).

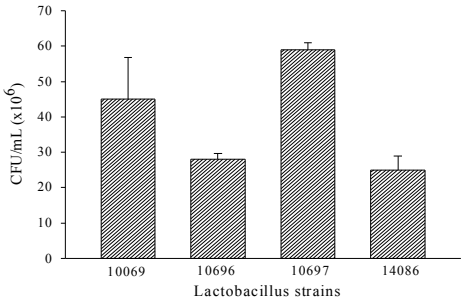

Fig. 1. Comparison of the total viable lactic acid bacteria among different fermented burdock with 48 h fermentation.

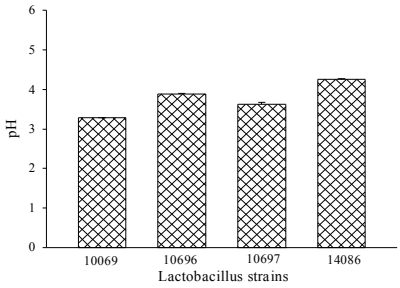

Fig. 2. Comparison of the pH among different fermented burdock with 48 h fermentation.

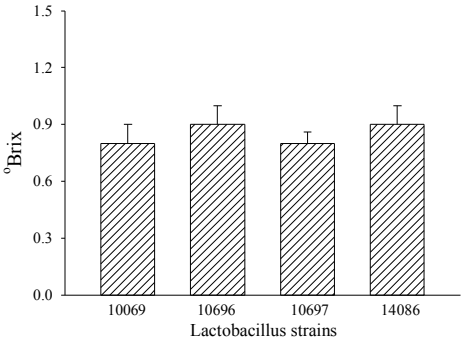

Fig. 3. Comparison of the sweetness among different fermented burdock with 48 h fermentation.

Burdock contains polyphenols such as chlorogenic acid, caffeic acid, isochlorogenic acid and dicaffeoylquinic acids which has been reported to have effect on anti-free radicals[8]. There are many diseases are associated with free radicals and consuming antioxidants food could reduce the incidence of the free radicals related diseases [23,24]. The present study is to further evaluate the functional ingredient and the free radical scavenging activity of burdock after fermentation with 14086, 10069, 10696 and 1069 strains at 37^{0}C for 48 hours. The result of the total polyphenols showed the total polyphenols is 10697≒10696≒14086＞10069 (Fig. 4). On DPPH free-radical scavenging ability, the result showed the DPPH free-radical scavenging ability of BFL is 10697≒10069＞14086＞10696 (Fig. 5).

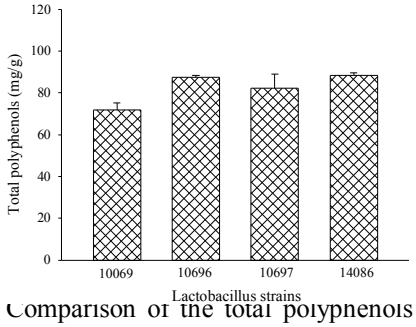

Fig. 4. Comparison of the total polyphenols among different fermented burdock with 48 h fermentation. Total polyphenols were expressed as mg gallic acid /g BFL.

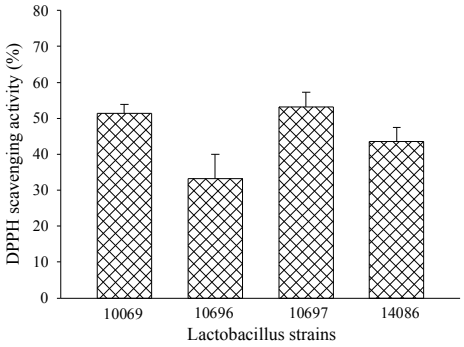

Fig. 5. Comparison of the free radical scavenging activity among different fermented burdock with 48 h fermentation.

In conclusion, the present study used four different lactobacillus to ferment burdock to explore the effect of different lactobacillus on functional ingredient and free radical scavenging activity of burdock. The overall results showed that lactobacillus 10697 strain has better effect on LBA populations, total polyphenols and free radical scavenging activities. The results can applied for developing burdock probiotic products with free radical scavenging activity.

Corresponding Author:
Fu-An Chen, Ph.D.
Department of Pharmacy & Graduate Institute of Pharmaceutical Technology,
Tajen University, 20 Wei-Shin Road, Yan-Pu, Pingtung, Taiwan, ROC.
E-mail: h56041@gmail.com

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