JOURNAL OF FUNCTIONAL FOODS 5 (2013) 905–913

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Anti-obesity activity of *Lactobacillus* fermented soy milk products

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ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form
31 January 2013
Accepted 31 January 2013
Available online 1 March 2013

Keywords:
Anti-obese activity
Lactobacillus paracasei subsp. *paracasei* NTU 101
Lactobacillus plantarum NTU 102
High-fat diet (HFD)
Daidzein
Genistein

ABSTRACT

The anti-obesity activity of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and *Lactobacillus plantarum* NTU 102 and their soy milk fermented products (SM101 and SM102) were investigated. Results indicated that the inhibition of 3T3-L1 differentiation and the accumulation of free fatty acids markedly increased in rats treated with SM101 and SM102. Moreover, the up-regulation and down-regulation of lipolysis and heparin-releasable lipoprotein lipase, respectively, were observed in the 3T3-L1 adipocytes of the SM101 and SM101 groups, and these effects of SM101 and SM102 were greater than unfermented soy milk (USM). We also found that SM101 and SM102 both improved obesity in Wistar rats fed with a high-fat diet (HFD) and that this improvement was stronger than that observed for USM. The level of serum leptin in HFD-induced rats was significantly elevated by the 5-week administration of SM101 and SM102 (10^6–10^{10} CFU/mL per rat per day); however, this activity was not promoted by USM. The anti-obesity activity of SM101 and SM102 may result from the increased daidzein and genistein levels that were observed during the fermentation by *L. paracasei* subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102.

1. Introduction

Lactic acid bacteria (LAB) are important members of the normal intestinal microflora and are reported to exert beneficial effects, including inhibition of the growth of potential pathogens, reduction of serum cholesterol, and modulation of the immune system (Tsai, Cheng, Fan, & Pan, 2008). In addition, they are used in the production of industrial chemicals, biological products, and food biopreservatives.

We have already screened two Taiwan native LAB strains: namely *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and *Lactobacillus plantarum* NTU 102 (Lin, Chiu, & Pan, 2004; Pan, Chiu, & Guu, 2002). We screened faeces and homemade Korean-style cabbage pickles which are resistant to gastric juice and bile salt in the natural environment. They also have "probiotic" characteristics that are effective in reducing cholesterol in the blood and liver (Chiu, Lu, Tseng, & Pan, 2006). After feeding mice with *L. paracasei* subsp. *paracasei* NTU 101, up-regulation of the antigen-presenting ability of dendritic cells and expression of natural killer group-2 D molecules that trigger NK-cell-mediated cytotoxicity were observed, and lymphocyte proliferation and antibody production were also significantly increased in mice after treatment (Tsai et al., 2008). On the other hand, soy skim milk fermented with *L. paracasei* subsp. *paracasei* NTU 101 and supplemented with or without *Momordica charantia* was found to be effective in preventing and slowing hyperlipidemia-induced oxidative stress and atherosclerosis (Tsai, Chu, Lee, & Pan, 2009). In addition, *L. plantarum* NTU 102 was beneficial for gastric mucosal lesions, increased antioxidant enzymes and phenol oxidase activities (Liu et al., 2009) and the immune response to *Litopenaeus vannamei* (Chiu, Gu, Liu, Pan, & Cheng, 2007).

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1756-4646/$ - see front matter
http://dx.doi.org/10.1016/j.jff.2013.01.040

We have also investigated the activities of *L. paracasei* subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102 on regulating blood pressure in spontaneously hypertensive rats (Liu et al., 2011). These studies reveal that both bacterial strains have potential for use in the development of functional fermented foods.

Metabolic syndrome is a group of conditions that increase the risk of cardiovascular disease and diabetes and are characterized by a group of metabolic risk factors, such as obesity, dyslipidemia, and elevated blood pressure. The beneficial effects of the oral administration of *Lactobacillus casei* on insulin resistance in diet-induced obesity mice has been reported in a recent study (Naito et al., 2011). On the other hand, several studies have reported that soy milk can also prevent obesity in high-fat diet (HFD)-induced animals (Choi et al., 2011; Eller & Deimer, 2010; Pimentel et al., 2012). However, the anti-obesity and hypolipidemic activities of *Lactobacillus* fermented soy milk in HFD-induced animals remain unknown. Therefore, this study was conducted to investigate the effect of *L. paracasei* subsp. *paracasei* NTU 101- and *L. plantarum* NTU 102-fermented soy milk on the prevention of obese in HFD-induced Wistar rats and to evaluate the activity of fermented products in inhibiting 3T3-L1 preadipocyte differentiation.

2. Materials and methods

2.1. Materials and methods

Cells 3T3-L1 preadipocyte was purchased from Bioresource Collection and Research Center (BCRC) in Hsinchu, Taiwan. Dulbecco's modified Eagle's medium and faetal bovine serum were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Dexamethasone, isobutylmethylxanthine, insulin, oil red O, heparin, crystal violet, and *p*-nitrophenyl butyrate were purchased from Sigma Chemical Co. (St Louis, MO, USA). Trypan blue stain was purchased from Gibco BRL Life Technologies Inc. (Gaithersburg, MD, USA). Penicillin and streptomycin were purchased from HyClone Laboratories (Logan, UT, USA). de Man, Rogosa and Sharpe medium (MRS) was purchased from Difco (Detroit, MI, USA).

2.2. Bacterial strains fermentation in soy milk

L. paracasei subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102 were used in this study. These strains were cultured on MRS medium. The statement reinoculation of MRS broth with 1% LAB were inoculated into 400 mL MRS broth and cultured at 37 °C for 24 h under aerobic conditions. The number and viability of the lactobacilli were determined by anaerobic cultivation on MRS plates (Liu et al., 2011). *L. paracasei* subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102 were grown in skim milk and soy milk (1:3, w/w) at 37 °C to ferment for 3 days. After fermentation, the product was freeze-dried.

2.3. Cell culture

3T3-L1 preadipocytes were cultured in basal medium (Dulbecco's modified Eagle's medium containing 10% fetal bovine serum) at 37 °C in 5% CO_2. To induce differentiation, 2-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 48 h with 0.5 μM methylisobutylxanthine, 1 μM dexamethasone and 10 μg/mL insulin (methylisobutylxanthine, dexamethasone, and insulin; MDI) added to basal medium. On day 2, the MDI medium was replaced with basal medium containing insulin only. On day 4 and thereafter, the cells were cultured in basal medium, which was freshly changed every 2 days until the cells were analyzed (Chen, Ho, Lee, & Pan, 2008).

2.4. Proliferation assay

3T3-L1 preadipocytes were seeded in 24-well dishes at a density of 7.5×10^3 cells per 0.5 mL per well. After the cells adhered to the dishes, samples were added to the culture medium at the indicated doses for 24 and 48 h. Viable cells at each dose and time point were stained for 10 min with 0.5% crystal violet, then dissolved in 2% sodium dodecyl sulphate (SDS) (w/v) after rinsed by H_2O, measured the optical density at A600 (Chen et al., 2008).

2.5. Lipolysis assay

The fully differentiated 3T3-L1 adipocytes (days 8–12 after differentiation induction) were treated with sample in Krebs Ringer bicarbonate (KRB) buffer (20 mM NaCl, 4.7 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgSO_4{\cdot}7H_2O$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 2% BSA; pH 7.4) for 24 h. Lipolysis activity was determined by measuring the amount of glycerol released into the incubation medium (GY105; Randox, San Diego, CA, USA). The cells were scraped off for measurement of the triacylglycerol (TG) content (Chen et al., 2008).

2.6. Assay for heparin-releasable lipoprotein lipase (HR-LPL activity)

The 3T3-L1 mature adipocytes were incubated with the experimental medium. Subsequently, the medium was discarded and the cells were rinsed with KRB buffer and then cultured in heparin-KRB buffer (10 U/mL heparin) at 37 °C for 1 h. The conditioned heparin-KRB was collected from each well for the assay of HR-LPL activity. LPL activity was measured on the basis of its esterase property using *p*-nitrophenyl butyrate as a substrate (Chen et al., 2008). The TG hydrolase activity of LPL with synthetic TG substrates is inhibited by sodium chloride (Shirai & Jackson, 1982), and this property has been used to distinguish LPL activity from the activities of other lipases in plasma. The HR-LPL activity was measured by the following equation: $C\ (\mu M) = (A_{400(0.15\ M\ NaCl)} - A_{400(1\ M\ NaCl)})/0.012$, and 0.012 is the micromolar extinction coefficient of *p*-nitrophenol.

2.7. Animals and diet

Male Wistar rats at 8 weeks-old (385 ± 14 g) were purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University (Taipei, Taiwan). Animals were provided with food and water ad libitum and subjected to 12 h light/dark cycle with a maintained relative humidity of 60% and a temperature at 25 °C. The experiments were carried