LWT - Food Science and Technology 85 (2017) 181–186

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Potential probiotic *Lactobacillus plantarum* Ln4 from kimchi: Evaluation of β-galactosidase and antioxidant activities

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ARTICLE INFO

Article history:
Received 8 February 2017
Received in revised form
11 July 2017
Accepted 11 July 2017
Available online 18 July 2017

Keywords:
Probiotic
β-Galactosidase
Antioxidant activity
DPPH radical-scavenging activity
β-Carotene oxidation-inhibitory activity

ABSTRACT

This study was conducted to evaluate the probiotic properties, including β-galactosidase and antioxidant activities, of lactic acid bacteria isolated from kimchi. Two isolates with a probiotic potential were isolated and identified by 16S rRNA sequence analysis. For comparison, a commercial probiotic strain, *Lactobacillus rhamnosus* KCTC 12202BP, was used. The isolates, *Lactobacillus plantarum* Ln4 and G72, and *L. rhamnosus* KCTC 12202BP, were able to survive under artificial gastric conditions (pH 2.5 in the presence of 0.3% pepsin and 0.3% oxgall). The safety of the LAB strains was tested in terms of antibiotic resistance and production of harmful enzymes. Antibiotic resistance was assessed according to Clinical and Laboratory Standards Institute guidelines. Assessment with the API ZYM kit showed that none of the strains produced harmful enzymes, such as β-glucuronidase. Among the tested strains, *L. plantarum* Ln4 showed the strongest adhesion to HT-29 cells and the highest β-galactosidase activity (3320.99 Miller Units). *L. plantarum* Ln4 was found to have higher 1-diphenyl-2-picrylhydrazyl radical-scavenging (40.97%) and β-carotene oxidation-inhibitory activities (38.42%) than did *L. rhamnosus* KCTC 12202BP. These results suggest that *L. plantarum* Ln4 isolated from kimchi may have a probiotic potential and could be used in functional foods.

1. Introduction

Kimchi, a traditional Korean fermented food made from Chinese cabbage and various seasonings such as radish, garlic, shrimp sauce, fish sauce, red pepper, and ginger, is widely recognized as a healthy food. Kimchi contains various biologically active components, including vitamins, chlorophylls, flavonoids, polyphenols, and lactic acid bacteria (LAB) (Jang, Chung, Yang, Kim, & Kwon, 2015). These components have various functional effects, such as antiobesity (Cui et al., 2015), antioxidant (Park et al., 2011), antimicrobial (Shin & Seo, 2003), and antitumor activities, among others. Kimchi contains various LAB strains, including *Leuconostoc* sp., *Lactobacillus* sp., *Lactococcus* sp., and *Weissella* sp. (Jung et al., 2011). The LAB strains that are present during kimchi fermentation are believed to have possible probiotic properties and health benefits (Ji et al., 2013).

Probiotics are defined as live bacteria that contribute to the regulation of immune responses and have beneficial effects on the host (Khan & Kang, 2016). A LAB strain can function as a probiotic if it is proven that the strain has some basic properties, including a high survival rate at low pH levels and in the presence of bile salts and strong adhesion to the cells of the intestinal tract. In addition, LAB strains produce beneficial enzymes, such as β-glucosidase, which converts glycosides into aglycones (Chun et al., 2007); β-galactosidase which hydrolyzes lactose (De Verse et al., 2003); and α-glucosidase, which breaks down starches and disaccharides into glucose and other products (Lee et al., 2014b). In addition, several researchers have reported that probiotics have antioxidant (Ji, Jang, & Kim, 2015), anticancer (Lee et al., 2015a), antiallergy (Lee, Kim, Han, Eom, & Paik, 2014a), and antimicrobial effects (Bao et al., 2010).

LAB have been continually investigated regarding the probiotic

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http://dx.doi.org/10.1016/j.lwt.2017.07.018
0023-6438/

potential, namely, the safety, stability, and functionality. Quality of fermented foods (kimchi, sauerkraut, jeotgal, and pickles) could be improved by LAB strains, for example, in terms of stability of quality, enhanced taste, and health-promoting benefits (Cui et al., 2015; Lee et al., 2014b; Lee et al., 2016). Especially, LAB strains isolated from kimchi can survive and persist in the intestinal tract because of their strong resistance to acid and bile salts (Lee et al., 2014b). Therefore, kimchi may serve as a source for selection of a potential probiotic because of harsh conditions and a large number of LAB strains in kimchi.

The aims of this study were to evaluate the probiotic properties and safety of LAB strains isolated from kimchi. In addition, β-galactosidase and antioxidant activities of the isolated strains were studied regarding possible functional effects.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Two strains, Ln4 and G72, were isolated from kimchi using lactobacillus selective agar (Difco Laboratories, USA), and were identified as *Lactobacillus plantarum* by 16S rRNA sequencing. *Lactobacillus plantarum* strains Ln4 and G72 were propagated and maintained in lactobacilli MRS (MRS) broth at 37 °C. *Lactobacillus rhamnosus* KCTC 12202BP, obtained from Cell Biotech (Korea), served as a commercial probiotic strain (control).

2.2. Tolerance to artificial gastric conditions

The tolerance of the isolated strains to artificial gastric conditions was determined as described by Lee et al. (2015b). Briefly, an overnight culture of the LAB strains was resuspended in the MRS medium (pH 2.5) containing 0.3% (w/v) of pepsin (Sigma, USA) and was incubated at 37 °C for 3 h. Viable cells were counted after plating on MRS agar.

To assess tolerance to bile salts, an overnight culture of each LAB strain was resuspended in the MRS medium containing 0.3% (w/v) of oxgall (Difco Laboratories, USA) and was incubated at 37 °C for 24 h. Viable cells were counted after plating on MRS agar.

2.3. Enzyme production

The API ZYM kit (BioMérieux, France) was employed to evaluate enzyme production. An overnight culture of each LAB strain was centrifuged (12,000 × *g*, 10 min, 4 °C), and the cell pellet was resuspended in sterile saline containing 0.85% sodium chloride (BioMérieux, France) at 10^5 colony-forming units (CFU)/mL and added to a culture. After the inoculation, the cultures were incubated for 4 h at 37 °C and then reagents zym A and zym B were added. Color intensity values (0–5) and substrate hydrolysis (in nanomoles) were determined.

2.4. Adhesion of LAB strains to HT-29 cells

A human colon adenocarcinoma cell line, HT-29, was obtained from the Korean Cell Line Bank (KCLB 30038; Korea). The cells were cultured in the RPMI 1640 medium (Hyclone, USA) containing 10% of fetal bovine serum (Hyclone) and 1% of a streptomycin/penicillin solution (Hyclone) at 37 °C in a humidified atmosphere containing 5% of CO_2.

The ability of LAB strains to adhere to HT-29 cells was determined as described by Lee et al. (2011), with minor modifications. For the adhesion assays, HT-29 cells were seeded in 24-well tissue culture plates at a concentration of 10^5 cells/mL. The LAB strains were added to the wells at approximately 10^7 CFU/mL and incubated for 2 h at 37 °C. After that, the monolayers were washed three times with sterile PBS (Hyclone, USA) to remove the non-adherent bacteria. Then, 1 mL of 1% (v/v) Triton X-100 (Sigma-Aldrich, USA) was added into each well and incubated for 10 min at 37 °C. After the incubation, the cells were detached from the well. Serial dilutions of the cell suspension were spread on MRS agar and incubated for 24 h at 37 °C to determine the percentage of viable cells. The adhesion ability of the LAB strains toward HT-29 cells was expressed as the percentage of viable bacteria remaining as compared to the initial bacterial counts added.

2.5. Antibiotic sensitivity of LAB strains

This property of the LAB strains was measured according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012). The disc diffusion method was applied to determine sensitivity to clinically important antibiotics, such as ampicillin (10 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), and doxycycline (30 μg). An aliquot of liquid culture of each LAB strain (10^6 CFU/mL) was spread on MRS agar, and paper discs containing the test antibiotics were placed on the plate. After incubation for 24 h at 37 °C, the diameters of the clear zones were measured.

2.6. β-Galactosidase activity of the LAB strains

This activity was measured according to a modification of a previously reported method (Vidhyasagar & Jeevaratnam, 2013). Briefly, the LAB strains were grown in MRS broth with or without lactose, centrifuged at 14,000 × *g* for 10 min at 4 °C, and washed twice with sterile PBS (Hyclone, USA). Each cell pellet was resuspended in Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, and 50 mM β-mercaptoethanol, pH 7.0). An aliquot of the cell culture (100 μL) was mixed with 900 μL of Z buffer, 0.1 mL of chloroform (John Baker, USA), and 0.05 mL of 0.1% SDS (Sigma-Aldrich, USA). Next, absorbance of the resuspended cells at 560 nm (blank, Z buffer) was measured. For the enzymatic assays, 900 μL of the cell suspension was mixed with 0.2 mL of Z buffer containing 4 mg/mL *ortho*-nitrophenyl-β-galactoside (Sigma-Aldrich) and was incubated at 37 °C for 15 min. To stop the reaction, 0.3 mL of 1 M sodium carbonate (Junsei, Japan) was added. Then, the reaction mixture was centrifuged at 12,000 × *g* for 10 min at 4 °C, and absorbance was measured at 420 and 550 nm. Enzymatic activity was calculated in Miller units according to the following equation:

$$\beta - \text{Galactosidase activity (Miller units, MU)} = 100 \times \frac{A_{420} - 1.75 \times A_{550}}{15\ \text{min} \times 1\ \text{mL} \times A_{560}}$$

2.7. Antioxidant activity of the LAB strains

2.7.1. Preparation of a cell-free supernatant

The LAB strains were grown in MRS broth for 20 h, centrifuged at 14,000 × *g* for 10 min at 4 °C, and washed twice with sterile PBS (Hyclone, USA). The washed bacterial cells were resuspended in PBS to a final concentration of 10^8 CFU/mL.

2.7.2. Free-radical-scavenging activity toward 1-diphenyl-2-picrylhydrazyl (DPPH)

Antioxidant activity was determined by the DPPH assay (Das & Goyal, 2015). An aliquot (100 μL) of the cell-free supernatant (10^8 CFU/mL) or PBS (control) was mixed with 100 μL of an