LWT - Food Science and Technology 71 (2016) 130–137

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Isolation of lactic acid bacteria with probiotic potentials from kimchi, traditional Korean fermented vegetable

Kang Wook Lee [a], Jae Min Shim [a], Seon-Kyung Park [a], Ho-Jin Heo [a, b], Hyun-Jin Kim [a, b], Kyung-Sik Ham [c], Jeong Hwan Kim [a, b, *]

[a] Division of Applied Life Science (BK21 Plus), Graduate School, Gyeongsang National University, Jinju 52828, Republic of Korea
[b] Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea
[c] Department of Food Science & Biotechnology and Solar Salt Research Center, Mokpo National University, Muan 58554, Republic of Korea

ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form
15 February 2016
Accepted 16 March 2016
Available online 19 March 2016

Keywords:
Lactic acid bacteria
Leuconostoc mesenteroides
Lactobacillus plantarum
Probiotic properties
HT-29 cells

ABSTRACT

Three *Leuconostoc mesenteroides* and a *Lactobacillus plantarum* strains were isolated from kimchi based on their acid and bile salts tolerances and bile salt hydrolase activities. They also possess β-galactosidase (β-Gal) and β-glucosidase (β-Glu) activities. When exposed to pH 3.0 for 3 h, *Lb. plantarum* C182 showed the highest survival ratio (52.50%). *Lb. plantarum* C182 was also the most resistant against 0.3% bile salts (58.53%) followed by *Leu. mesenteroides* C10 (42.85%), F27 (41.17%) and C4 (30.41%). All strains were sensitive to ampicillin, chloramphenicol, cyclohexamide, erythromycin, neomycin, streptomycin, tetracycline, and rifampicin, but resistant to vancomycin. Three *Leuconostoc* strains adhered to HT-29 cells (16.3–29.6 CFU/cell) better or similar with *Lb. rhamnosus* GG (17.1 ± 0.6 CFU/cell). These results indicate that 4 strains might be useful as probiotics.

1. Introduction

Probiotics are live microorganisms which confer health-promoting benefits to hosts by maintaining or improving the intestinal microbial biota (Asahara et al., 2004; Ouwehand, Kirjavainen, Shortt, & Salminen, 1999). If microorganisms are used as probiotics, they should possess some properties such as an ability to strongly adhere to epithelial cells in the gastrointestinal tracts of hosts and to survive under unfavorable conditions including low pHs and bile salts. In addition, probiotics should be sensitive to antibiotics used for the treatments of infections. Possession of some desirable enzyme activities such as β-galactosidase (β-Gal) and β-glucosidase (β-Glu) is another advantage because β-Gal alleviates lactose intolerance (de Verse et al., 2003) and β-Glu liberates bioactive aglycones from isoflavone glucosides (Chun et al., 2007). Microorganisms belonging to the genera of *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Bifidobacterium* are the most common probiotics.

Kimchi is a Korean traditional fermented vegetable, fermented mainly by various lactic acid bacteria (LAB) after brined cabbage or radish was mixed with various spices such as red pepper, garlic, ginger, green onion and other ingredients (Jung, Lee, & Jeon, 2014; Park, Jeong, Lee, & Daily, 2014). Kimchi fermentation is carried out at low temperatures, usually below 10 °C and various LAB proliferate during kimchi fermentation, contributing to the development of unique taste and flavor of kimchi (Cheigh & Park, 1994; Kwon & Kim, 2007). *Leuconostoc mesenteroides* is the most important and the major organism during the early and middle stages of kimchi fermentation (Chang & Chang, 2010; Jung et al., 2014). During the middle and later stages of fermentation, lactobacilli such as *Lactobacillus plantarum* become dominant, producing more acids and resulting in acidification of kimchi (Lee et al., 2015). Due to the combined effects of ingredients and LAB, kimchi is well-known as a healthy food. Some health-promoting effects including anticancer, antioxidative, antidiabetic and antiobesity effects have been reported (Islam & Choi, 2009; Park et al., 2014).

Traditionally, kimchi has been prepared by natural fermentation, without using starters. However, in recent days, selected LAB are being used as starters for kimchi for the purpose of improving the quality, functionality, and extending the shelf-life of kimchi (Chang & Chang, 2011; Jung et al., 2012; Lee et al., 2015; Seok et al., 2008). Ideal kimchi starters should possess properties required for

* Corresponding author. Division of Applied Life Science, Graduate School (BK21 Plus) Gyeongsang National University, Jinju 52828, Republic of Korea.
E-mail address: jeonghkm@gnu.ac.kr (J.H. Kim).

http://dx.doi.org/10.1016/j.lwt.2016.03.029
0023-6438/

ideal probiotics since kimchi is consumed daily without heat treatments and thus a large number of LAB enter into human body. In this study, we examined the properties of 4 LAB isolated from kimchi and found that they can be suitable as kimchi starters and potential probiotics.

2. Materials and methods

2.1. Isolation and identification of LAB strains

LAB were isolated from kimchi. Kimchi samples (cabbage kimchi) were purchased at local markets at Jinju, Gyeongnam, Republic of Korea during the summer season in 2014. Kimchi samples (20 g) were mixed with 80 ml of 0.1% peptone water and homogenized with Stomacher 80 (Seward, Worthing, UK). Serially diluted kimchi samples were spread on deMan Rogosa Sharpe (MRS, Difco, Becton Dickinson Co., Sparks, MD, USA) agar plates and incubated at 30 °C statically. Isolated strains were examined for their acid tolerance, bile salts tolerance, and activities of bile salt hydrolase and other useful enzymes. For tolerance against acid and bile salts, the overnight cultures of isolates were spotted on MRS agar plates adjusted to pH 2.0 or MRS agar plates supplemented with 0.3% bile salts (cholic acid sodium salt 50% and deoxycholic acid sodium salt 50%, Sigma–Aldrich, 48305). For enzyme activities, the overnight cultures were spotted on MRS agar plates supplemented with 0.5% (w/v) taurodeoxycholic acid (Sigma) or 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) or 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside (X-α-Gal). The plates were incubated for 24 h at 30 °C. The presence of each enzyme activity was visualized as an altered colony morphology (clear zone around the colony and color of the colony) as compared with the negative control (*Lactococcus lactis* MG1363). Identification of selected strains was done by using API 50CHL kit (BioMerieux, Marcy L'Etoile, France) and 16S rRNA gene sequencing. The universal primers, 27F and 1492R, were used to amplify 16S rRNA genes: 27F (5′-AGAGTTTGAT CMTGGCTCAG-3′) and 1492R (5′–TACGGYTACCTTGTTAC GACTT-3′) (Lee et al., 2012). Amplified fragments were examined by agarose gel electrophoresis and purified using a PCR purification kit (FavorPrep PCR purification mini kit, Favorgen, Ping-Tung, Taiwan). The sequences were determined at Cosmogenetech (Seoul, Korea) and BLAST program was used to find homologous sequences in the database (http://www.ncbi.nlm.nih.gov/BLAST).

2.2. Growth of isolated strains

Isolated strains were grown for 16 h in MRS broth at 30 °C without shaking and then inoculated (1%, v/v) into 50 ml of fresh MRS broth with different initial pH (2–9) and salt concentration (6.5 and 8%, w/v). Inoculated cultures were incubated for 48 h or 96 h and the OD_{600} values of cultures were measured during cultivation. Strains were also grown in regular MRS broth at different temperature.

2.3. Enzyme activities of isolated strains

API ZYM system was used to evaluate the enzyme activities of isolated strains (Humble, Anna, & Phillips, 1977). β-Gal activity was measured using the method of Miller (1972). Cells were collected by centrifugation and resuspended in 1 ml of Z buffer (60 mM Na_2HPO_4 $7H_2O$, 40 mM NaH_2PO_4 H_2O, 10 mM KCl, 1 mM $MgSO_4$ $7H_2O$, 50 mM β-mercaptoethanol, pH 7.0), placed on ice and disrupted by sonication (30 s, 6 times) using a homogenizer (Bandelin, Berlin, Germany). Disrupted cells were incubated with 200 ml of *O*-nitrophenyl-β-D-galactopyranoside (ONPG) solution (4 mg/ml in A buffer; K_2HPO_4 1.05 g, KH_2PO_4 0.45 g, $(NH_4)_2SO_4$ 0.1 g, Na_3-citrate-2 H_2O 0.05 g, 100 ml final volume) at 28 °C until yellow color appeared. Reaction was stopped by the addition of 1 M sodium carbonate. After centrifugation at 12,000*g* for 10 min, the absorbance of supernatant was measured at 420 nm and 550 nm.

β-Glu activity was determined by the method of Belancic, Gunata, Vallier, and Agosin (2003) using *p*-nitrophenyl-β-D-glucopyranoside (pNPG) as the substrate. After cells were disrupted by sonication (30 s, 6 times), β-Glu activity of cell extract was determined by incubating mixture consisting of 50 ml of pNPG (10 mM), 50 ml of sodium acetate (200 mM, pH 5.5) and 0.1 ml of cell extract for 15 min at 37 °C. The reaction was stopped by the addition of 800 ml of 1 M sodium carbonate. The amount of *p*-nitrophenol (pNP) released was measured at 400 nm using a spectrophotometer. One unit of β-Glu activity was defined as the amount of enzyme that released 1 μmol of pNP from the substrate per min.

α-Galactosidase (α-Gal) activity was measured by method of Church, Meyers, and Srinivasan (1980). Cells were disrupted by sonication (30 s, 6 times). *p*-nitrophenyl-α-galactopyranoside (pNPGal) was used as the substrate and the released pNP was measured at 400 nm α-Glucosidase (α-Glu) activity was measured by using *p*-nitrophenyl-α-D-glucopyranoside as the substrate (Bergmeyer, Graβl, & Walter, 1988). Enzymatic reaction was performed by mixing 0.9 ml of 1 mM substrate prepared in 50 mM sodium phosphate buffer (pH 6.5) with 0.1 ml of cell extract and incubated at 37 °C. The amount of pNP released was examined by measuring absorbance at 400 nm.

2.4. Acid resistance and bile salt tolerance of isolated strains

Isolated strains were cultivated in MRS broth until the OD_{600} reached 1.2. One ml of culture was centrifuged at 12,000*g* for 10 min at 4 °C and cell pellet was resuspended in MRS broth where the pH was adjusted to 2 or 3 or 6.5 (control), respectively, by 1 N HCl. Cell suspensions were incubated for 3 h at 37 °C and then viable cells were counted by standard plate counting. Measurements were done in triplicates and the mean values were shown.

Bile tolerance of isolated strains was examined. Strains were cultivated in MRS broth until the OD_{600} reached 1.2 and cells were harvested by centrifugation (12,000g, 10 min, 4 °C). Cell pellet was washed with sterile water and resuspended in 1 ml of MRS broth containing 0.3% bile salts (cholic acid sodium salt 50% and deoxycholic acid sodium salt 50%, Sigma–Aldrich, 48305). After 3 h incubation at 37 °C, viable cells were counted. All measurements were repeated three times (three separate samples) with duplicate determinations and the results were shown as mean ± SD (standard deviation).

2.5. Resistance to antibiotics of isolated strains

Antibiotic susceptibilities of isolated strains were examined by agar overlay diffusion method as described by Cebeci and Gürakan (2003) with some modification. MRS agar plates were overlaid with 0.7% soft agar with 200 μl of each culture (10^8 CFU/ml), grown overnight in MRS broth at 30 °C, and stood for 1 h at 30 °C. Then paper discs were placed and antibiotics were applied onto the discs. After 24 h incubation at 30 °C, the diameters of inhibition zones were measured. The strains were tested for their susceptibilities against ampicillin, chloramphenicol, cyclohexamide, erythromycin, neomycin, streptomycin, spectinomycin, tetracycline, rifampicin and vancomycin. Antibiotics were used at the concentration of 0.5–2048 mg per disc.