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Functional properties of *Lactobacillus* strains isolated from kimchi

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ABSTRACT

The objective of this study was to evaluate the functional properties of lactic acid bacteria (LAB) from kimchi, a traditional Korean fermented vegetable product generally consumed raw as a side-dish with practically every meal.

Twelve mild acid producing facultatively heterofermentative Lactobacillus strains were selected for their potential as starter cultures for fermentation of kimchi, and evaluated for their functional properties. Eleven strains were identified as *Lactobacillus sakei* and one as *Lactobacillus plantarum*. The strains identified as *L. sakei* differed in some physiological features; of particular interest was the fact that 9 of these strains produced L(+) lactic acid from glucose in presence of acetate.

All strains were able to survive gastrointestinal conditions simulating stomach and duodenum passage. In addition, they showed higher adherence to HT-29 cells than *Lactobacillus rhamnosus* GG, a commercial probiotic strain used worldwide. These strains also showed antimicrobial activity against a number of food-borne pathogens. Their ability to lower cholesterol was demonstrated by BSH (bile salt hydrolytic) activity, and cholesterol assimilation tests *in vitro*. The results suggest the probiotic potential of these strains for use in kimchi fermentation.

1. Introduction

Kimchi is a Korean traditional fermented vegetable product and is gaining popularity as a functional food. It is typically consumed in a raw (uncooked) state, and serves as a side-dish with practically every meal. The magazine *Health* mentioned kimchi in its list of the top five "World's Healthiest Foods" (http://eating.health.com/2008/02/01/worlds-healthiest-foods-kimchi-korea/#). The beneficial effects of kimchi on human health may be derived from nutrients and functional components in kimchi such as vitamins, minerals, fibers and phytochemicals, from the biological compounds present either in kimchi ingredients such as garlic, ginger, red pepper powder, the fermentation products and the lactic acid bacteria (LAB) involved, or a combination of these. Functional properties of kimchi responsible for the health benefits have been claimed, amongst others, as anti-oxidative activity (Hwang et al., 2000; Lee et al., 2004; Ryu et al., 2004), anti-aging effects (Kim et al., 2000, 2002), anti-mutagenic and anti-tumor activities (Cho et al., 1997; Park, 1995; Shin et al., 1998), anti-microbial activity (Sheo and Seo, 2003), immune stimulatory activity (Kim et al., 1997), weight-controlling activity, lipid-lowering activity, and anti-atherogenic activity (Kim and Lee, 1997; Kim et al., 2004; Kwon et al., 1999; Sheo and Seo, 2004). Kimchi, being a lactic acid-fermented vegetable product consumed raw, is therefore also considered to be a good source of potentially beneficial and useful LAB. Among these, particularly the lactobacilli are of interest as beneficial inhabitants of the intestinal tract of man and animals. A wide range of such desired roles have been suggested for diverse strains of LAB, including immune stimulation, pathogen exclusion, production of bioactive substances, and general intestinal health.

The over-ripening (or over-souring) of kimchi is the most serious defect in kimchi, and is mainly due to the overproduction of lactic acid by some strains of LAB. Still, overall, the LAB play an important role in the taste and quality of kimchi, and many strains associated with kimchi show antimicrobial activity in addition to other useful properties. The best way to overcome the problem of excessive acid production is to control the growth of LAB without harming the quality of the end product.

The major objective of the reported work was to study the functional properties of LAB from kimchi. Although the technical attributes of strains are also of importance, the focus of this work was primarily on potential health benefits of mild acid producing strains, thereby contributing to a product of overall good quality. The ultimate goal would be to apply such “multifunctional” strains as starter cultures to control the growth of high acid producing LAB strains without reducing the product quality, and, at the same time, to contribute to the health related functional properties of the final product.

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2. Materials and methods

2.1. Strains

Lactic acid bacterial strains were isolated from home-made and commercial kimchi using MRS agar for plating. Representative colonies were picked from plates with the highest dilution still showing colonies. After purification, the strains were stored at -80 °C in MRS broth with 20% glycerol. The stock cultures were propagated twice in MRS broth for 18 h before each experiment.

Reference strains were obtained from the ATCC (American Type Culture Collection, Manassas, VA, 20110-2209, USA), KACC (Korean Agricultural Culture Collection, National Institute of Agricultural Biotechnology, 225 Seodun-dong, Suwon, Gyunggi Province, 441-707, Republic of Korea), and KCTC (Korean Collection for Type Cultures, Daejon 305-333, Republic of Korea).

Strains of food-borne pathogens and LAB were propagated twice in LB (Luria-Bertani) broth (Difco, USA), BHI (Brain Heart Infusion) broth (Difco, USA), and MRS broth (Difco, USA) for 18 h at 37 °C before each experiment.

Test strains: *Listeria innocua* KACC 3586, *Listeria monocytogenes* KCCM 40307, *Bacillus cereus* ATCC 08715, *Staphylococcus* aureus KCCM 11335, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Weissella koreensis* KACC 11853.

2.2. Physiological properties

2.2.1. Carbohydrate fermentation

Carbohydrate fermentation was determined as described by Mathara et al. (2004). Strains were grown overnight at 30 °C in MRS and the cell pellet collected by centrifugation at 10,000×*g* for 5 min. The pellet was washed twice in sterilized double distilled water before re-suspending it in basal medium. The first determination of the carbohydrate fermentation profile of all the strains was done in MRS fermentation broth without glucose but with chlorophenol red (0.04 g/l) as pH indicator. The individual sugars were prepared as 2.5% (m/v) solutions and filter sterilized using a 0.45 μm filter. A microtiter plate was used whereby 25 μl of sugar solution was added to 100 μl of basal medium containing the washed cell suspension.

2.2.2. Hydrolysis of Arginine

The experiment for the production of ammonia from arginine was carried according to Harrigan and McCance (1976). MRS broth without ammonium citrate was used as growth medium.

2.2.3. Dextran production

The strains were streaked onto MRS agar supplemented with 10% sucrose (Junsei, Japan). After 24 - 48 h incubation at 30 °C, colonies were investigated for dextran (slime) production. Non-producers showed no change, and had normal shape and color.

2.2.4. Presence of haem-dependent catalase activity

Cells were grown aerobically at 30 °C in MRS broth containing 5 g glucose/l (Sigma, USA) supplemented with 30 μg haematin (Sigma, Switzerland). After 24 h, catalase activity was determined by the presence of effervescence when adding 0.6% H_20_2 (Berthier and Ehrlich, 1999).

2.2.5. Determination of lactic acid enantiomers produced

Lactic acid enantiomers were determined using an enzymatic kit (r-biochem, Germany) based on the oxidation of D(-)-lactate and L(+)-lactate to pyruvate in the presence of NAD+, which in turn reduces to NADH by the corresponding enzymes D-lactate-dehydrogenase or L-lactate-dehydrogenase. Strains were grown at 30 °C for 18-24 h in MRS broth with and without sodium acetate.

2.2.6. Detection of meso-diaminopimelic acid (m-DAP)

Presence of *meso*-DAP in the bacterial cell wall was determined using thin-layer chromatography on cellulose plates as described by Tamang et al. (2000).

2.3. Determination of functional properties for selection of strains

2.3.1. Response to simulated stomach duodenum-passage (SSDP)

This assay was designed to represent a simplified and standardized test system giving predictive values for the assumed survival of LAB in the human stomach and duodenum under simulated physiological conditions as described by Haberer et al. (2002).

2.3.2. Determination of cell surface hydrophobicity

The test for bacterial adhesion to hydrocarbon was adopted to screen lactobacilli for cell surface hydrophobicity (Doyle and Rosenberg, 1995). For hydrophobicity testing, bacteria were grown in MRS broth at 37 °C for 18 h. Cells from 5 ml of culture were collected by centrifugation (9500×*g*) at 4 °C for 6 min. The cells were washed twice with PBS. One ml of this suspension was used to determine the OD_{580}. In duplicate assessments, a further 1.5 ml of this suspension was added to an equal volume of n-hexadecane (Sigma, USA) and thoroughly mixed for 2 min using a vortex. The phases were allowed to separate at room temperature for 30 min, after which one ml of the watery phase was removed and the OD_{580} was determined. The OD_{580} of duplicate assessments was meaned and used to calculate the degree of hydrophobicity. Percentage hydrophobicity was calculated as follows: (OD_{580} reading 1- OD_{580} reading2/ OD_{580} reading 1)×100 = % hydrophobicity.

2.3.3. Adhesion properties to human cell lines (HT29 cell cultures)

Mucus secreting HT29 cells were routinely grown in RPMI 1640 medium (Hyclone Laboratories Inc., USA), supplemented with 10% bovine calf serum (BCS), (Hyclone, New Zealand) and 100 μg streptomycin and 100 U penicillin per ml (Hyclone Laboratories Inc., USA) at 37 °C in a 5% CO_2 atmosphere. For adhesion assays, HT29 monolayers were prepared in 24-well Costar® tissue plates (Corning Incorporated, USA) according to Schillinger et al. (2005). Cells were inoculated at a concentration of 5.4×10^5 cells per well to obtain confluence and cultured for 21 days prior to the adhesion assay. The cell culture medium was changed on alternate days, and the last two media changes were without penicillin/streptomycin.

2.3.3.1. In vitro adherence assay. Strains in this assay were selected on the basis of their hydrophobic characteristics and their ability to survive acid and bile environments. The probiotic strain *Lactobacillus rhamnosus* GG was used as control.

2.3.4. Agar diffusion method for antibiotic resistance

The tests performed according to according to Clinical and Laboratory Standards Institute guidelines (CLSI) formerly NCCLS (NCCLS, 1997). For the assessment of the susceptibility to antimicrobials of bacterial strains, serial two-fold dilution procedures in agar was used and include *Lactobacillus plantarum* 299v as a relevant probiotic control strain. Test strains were grown for 18 hours at 37 °C in the MRS and then these cultures were diluted with the same fresh medium to a density 10^5 CFU/spot. Plates were incubated in air at 37 °C for 48 h and were examined for growth. Minimum inhibitory concentration (MIC) was considered as the lowest concentration of the antimicrobial that inhibits bacterial growth.

2.3.5. Detection of biogenic amine production

The decarboxylase detection media and procedures of Bover-Cid and Holzapfel (1999) were used. All strains were subcultured 5 to 10 times in MRS broth containing 0.1% of each precursor amino acid (L-Tyrosine disodium salt, L-Histidine monohydrochloride monohydrate,