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Research article

Evaluation of ginsenoside bioconversion of lactic acid bacteria isolated from kimchi

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ABSTRACT

Background: *Panax ginseng* is a physiologically active plant widely used in traditional medicine that is characterized by the presence of ginsenosides. Rb1, a major ginsenoside, is used as the starting material for producing ginsenoside derivatives with enhanced pharmaceutical potentials through chemical, enzymatic, or microbial transformation.

Methods: To investigate the bioconversion of ginsenoside Rb1, we prepared kimchi originated bacterial strains *Leuconostoc mensenteroides* WiKim19, *Pediococcus pentosaceus* WiKim20, *Lactobacillus brevis* WiKim47, *Leuconostoc lactis* WiKim48, and *Lactobacillus sakei* WiKim49 and analyzed bioconversion products using LC-MS/MS mass spectrometer.

Results: *L. mesenteroides* WiKim19 and *Pediococcus pentosaceus* WiKim20 converted ginsenoside Rb1 into the ginsenoside Rg3 approximately five times more than *Lactobacillus brevis* WiKim47, *Leuconostoc lactis* WiKim48, and *Lactobacillus sakei* WiKim49. *L mesenteroides* WIKim19 showed positive correlation with β-glucosidase activity and higher transformation ability of ginsenoside Rb1 into Rg3 than the other strains whereas, *P. pentosaceus* WiKim20 showed an elevated production of Rb3 even with lack of β-glucosidase activity but have the highest acidity among the five lactic acid bacteria (LAB).

Conclusion: Ginsenoside Rg5 concentration of five LABs have ranged from ~2.6 μg/mL to 6.5 μg/mL and increased in accordance with the incubation periods. Our results indicate that the enzymatic activity along with acidic condition contribute to the production of minor ginsenoside from lactic acid bacteria.

1. Introduction

Lactic acid bacteria (LABs) have been used as probiotics and are present in many fermented foods (cheese, yogurt, butter, and kimchi), where they influence the taste and preservation by producing lactic acid and/or alcohol. Some enzymes produced by LABs can efficiently utilize ingested nutrients to benefit the host, i.e., linoleic acid isomerase from *Lactobacillus acidophilus* produces conjugated linoleic acid, which has biological properties, from linoleic acid [1], and β-glucosidase from *Lactobacillus paraplantarum* converts isoflavone glucosides, which are not absorbed by enterocytes [2], to absorbable aglycones [3]. Recent studies reported the bioconversion of ginsenosides using *Lactobacillus pentosus* and *Leuconostoc citreum* isolated from fermented foods due to β-glucosidase activity [4,5].

The major commercial ginsengs such as *Panax ginseng* Meyer (Korean Red Ginseng), *Panax quinquifolium* (American ginseng), and *Panax notoginseng* (Burk.) F.H. Chen (Notoginseng) have been widely used as traditional herbal medicines [6]. Ginsenosides (ginseng saponins) are the major pharmacological constituents of ginseng, and over 100 ginsenosides have been identified [5,7]. Major ginsenosides (80% of the ginsenosides) are composed of Rb1, Rb2, Rc, Rd, Re, and Rg1; minor ginsenosides are their deglycosylated forms and composed of Rg3, Rh2, Rh1, F2, C-K, Rg2, Rh1, Rg5, and F1 [8].

Minor ginsenosides are known to have a greater pharmaceutical potential than major ginsenosides [9–14]. However, naturally occurring minor ginsenosides are present at very low concentrations. Therefore, hydrolysis of sugar moieties from abundant major ginsenosides are needed to produce minor ginsenosides. Gut

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microbiota metabolize orally administered ginseng and help transport across the epithelial membrane [15] and human intestinal microbiota convert major ginsenosides to minor ginsenosides [10,16,17]. However, ginsenoside metabolism varies between individuals depending on the population of gut microbiota, such as *Ruminococcus* spp., *Bacteroides* spp., and *Bifidobacterium* spp. [17]. Acidic environments as well as intestinal microbiota have important influences on the bioconversion of ginsenoside and the low pH of gastrointestinal environment could activate the deglycosylation of ginsenoside by acidic hydrolysis response [18–20].

Kimchi is a traditional Korean food that is fermented vegetables including cabbage and various seasonings. Kimchi has antioxidative and antidiabetic properties and bacteria isolated from kimchi produce beneficial enzymes [21–23]. Various LABs play important roles during kimchi fermentation: *Lactobacillus* and *Leuconostoc* are the predominant genera of the kimchi microbiome in the kimchi fermentation [24]. *Lactobacillus* species have neuroprotective, antifungal, and anticolitic properties [25–27] and *Leuconostoc* species play key roles in decreasing foodborne pathogen growth, viral activity, and the effects of lipid profiles [28–30]. Recent studies have suggested that LABs from kimchi produce hydrolytic enzymes that catalyze ginsenoside bioconversion by removing the glycosyl group of major ginsenosides [4,5].

In this study, we isolated LABs associated with kimchi fermentation from homemade kimchi, and compared availability for ginsenoside bioconversion of five LAB strains such as *Leuconostoc mensenteroides* WiKim19, *Pediococcus pentosaceus* WiKim20, *Lactobacillus brevis* WiKim47, *Leuconostoc lactis* WiKim48, and *Lactobacillus sakei* WiKim49 by quantitating transformed ginsenoside using a sensitive and reliable LC-MS/MS method.

2. Materials and methods

2.1. Materials

Leuconostoc mensenteroides WiKim19, *Pediococcus pentosaceus* WiKim20, *Lactobacillus brevis* WiKim47, *Leuconostoc lactis* WiKim48, and *Lactobacillus sakei* WiKim49 were isolated from homemade kimchi using de Man, Rogosa, and Sharpe (MRS) media. MRS broth was purchased from Difco (Miller, Becton Dickinson, and Co., Sparks, MD, USA). Ginsenosides Rb1, Rg3, digoxin (internal standard), and β-glucosidase activity assay kit were purchased from Sigma Aldrich (St. Louis, MO, USA). Ginsenoside -F2, -Rg1, -Rf, -Ro, -Rg2, -R1, -Ra1, -Rb2, -Rb3, -F1, -Rd, -Rg5, -compound K; -Rh2, -Rh4, and gypenoside XVII were purchased from Ambo Institute (Daejeon, Korea). API 50 CH and inoculating fluid was purchased from bioMérieux (Lyon, France).

2.2. Determination of 16S rRNA gene sequences and phylogenetic analysis

To identify the isolates using 16S rRNA sequencing, the isolates were sent to Macrogen Inc., Korea sequencing service (www.macrogen.com). The obtained sequences were compared with available 16S rRNA sequences in the EzTaxon Server [26] to evaluate sequence similarity. Multiple sequence alignment of the 16S rRNA sequences from five lactic acid bacteria and these related species were performed with CLUSTAL W [27]. The phylogenetic trees were constructed using MEGA6 [28] with neighbor-joining [29] based on 1,000 random bootstrap replicates for each.

2.3. Assay of ginsenoside Rb1 bioconversion by lactic acid bacteria

L. mensenteroides WiKim19, *P. pentosaceus* WiKim20, *L. brevis* WiKim47, *L. lactis* WiKim48, and *L. sakei* WiKim49 were inoculated in MRS broth, until absorbance reached 600 nm of 1.0. The strains were cultured at 30°C for 1 d, 3 d, and 7 d with ginsenoside Rb1 (a final concentration of 200μM) dissolved in MeOH. After centrifugation at 5,000*g* for 10 min, 2 mg/mL digoxin as an internal standard was added and purified using Sep-Pak Light C18 cartridges (Waters, Milford, MA, USA) and then dissolved in MeOH.

2.4. Assay of ginsenosides by LC-MS/MS

Ginsenosides Rb1 and minor ginsenosides in the reactions were analyzed by using UPLC (Waters), coupled to a TripleTOF 5600 plus system with electrospray ionization (ESI; AB SCIEX, Framingham, MA, USA). To investigate and separate the precursor and fragmentation ions of ginsenosides Rb1, minor ginsenosides, an Acquity UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm particle size) from Waters was used at a flow rate of 0.5 mL/min. UPLC conditions were as follows: solvent A, water containing 10mM ammonium acetate; solvent B, acetonitrile containing 10mM ammonium acetate; gradient, 0–0.5 min (5% B), 0.5–14.5 min (5–30% B), 14.5–15.5 min (30–32% B), 15.5–16.5 min (32–40% B), 16.5–17 min (40–55% B), 17–19 min (55% B), 19–25 min (90% B), and 25–30 min (5% B). Two microliters of each sample were injected for the UPLC analysis, and peaks were identified by comparing their retention times and fragment ion with that of reference compound.

The mass spectrometry conditions were optimized under the negative ion mode as follows: curtain gas, 30; collision energy, −30; declustering potential, −80; nebulizer gas (Gas 1), 40 at MRM mode; heater gas (Gas 2), 50. The ion spray voltage was −4,500 V. Ginsenosides in all reaction mixtures were quantified with multiple reaction monitoring (MRM) using selected transitions as follows: Rb1, *m/z* 1,107→945; Rg3, F2, *m/z* 783→621; Rg5, *m/z* 765→603; digoxin, *m/z* 779→649, Rg1, Rf *m/z* 799→637; Ro, m/z 955 → 793; Rg2, *m/z* 783→637; R1, *m/z* 931→769; Ra1, *m/z* 1,209→1,077; Rb2, Rb3, *m/z* 1,077→ 945; F1, *m/z* 637→475; Rd, *m/z* 945→783; XVII, *m/z* 945→323; compound K, *m/z* 621→459; Rh2, *m/z* 621→459; and Rh4 *m/z* 619→161.

Data acquisition and processing were carried out using Analyst TF 1.6 and PeakVeiw 1.2 software (AB SCIEX), respectively. The data obtained from multiple reaction monitoring (MRM) mode were quantitated using MultiQuant software (AB SCIEX). The standard solutions containing 10–200μM were injected into the UPLC with 2 mg/mL digoxin. The linear calibration curve for peak area ratio (ginsenoside/digoxin) was obtained for the quantification of ginsenoside. The amounts of the ginsenosides in each sample were determined from corresponding calibration curves.

2.5. Assay of β-glucosidase activities using cell lysates

L. mensenteroides WiKim19, *P. pentosaceus* WiKim20, *L. brevis* WiKim47, *L. lactis* WiKim48, and *L. sakei* WiKim49 were cultured for 1 d in MRS broth at 30°C. The supernatant was removed after centrifugation at 12,000*g* for 10 min, and cell lysates including intracellular β-glucosidase were prepared by bead beating in 50mM sodium phosphate buffer (pH 7.0). Protein concentrations of cell lysates were determined using a Pierce BCA Protein Assay Kit (Thermo, Rockford, IL, USA). The proteins were diluted to the concentration of ∼0.5 mg/mL to assay enzyme activity. The enzyme activity was determined using β-glucosidase activity assay kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol. The release of *p*-nitrophenol was measured at 405 nm (SPECTROstar Nano, BMG Labtech, Ortenberg, Germany). Analysis was performed in duplicate for each strain. One unit of β-glucosidase is the amount of enzyme that catalyzes the hydrolysis of 1.0 μmole substrate per min at pH 7.0.

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