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An improved process of isomaltooligosaccharide production in kimchi involving the addition of a *Leuconostoc* starter and sugars

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ABSTRACT

Isomaltooligosaccharides (IMOs) are α-(1 → 6)-linked oligodextrans that show a prebiotic effect on *Bifidobacterium* spp. This study sought to improve IMO synthesis during lactate fermentation in kimchi by inoculating the kimchi fermentation mix with a starter and sugars; the psychrotrophic *Leuconostoc citreum* KACC 91035 strain with high dextransucrase activity was used as a starter and sucrose (58 mM) and maltose (56 mM) were added as the donor and acceptor for the glucose-transferring reaction of the dextransucrase, respectively. With the addition of both the starter and the sugars and incubation at 10 °C, IMOs were produced in kimchi after 3 d. Without the starter, the IMO production rate and maximal concentration in kimchi were 15.05 mM/d and 75.27 mM, respectively, whereas with the starter, the rate and concentration increased to 22.04 mM/d and 110.19 mM, respectively. In addition, the sucrose–maltose mix gave an appropriate level of sweetness by releasing fructose and prevented unfavorable polymer synthesis by IMO production. This result suggests that lactic acid bacteria expressing a highly active glycosyltransferase can be used for the synthesis of beneficial oligosaccharides in various fermented foods.

1. Introduction

Lactic acid fermentation of vegetables, a valuable technique used by humans for thousands of years, is still investigated today because of the nutritional value of its products, which provide vitamins, minerals, and dietary fiber, and help the intestinal tract maintain a healthy balance of flora by increasing beneficial intestinal bacteria (Steinkraus, 1983). Among the diverse commercial products globally derived from fermented vegetables, kimchi made from cabbage is most economically relevant (Caplice and Fitzerald, 1999). Kimchi is a traditional Korean food produced by the fermentation of *baechu*-cabbage and other ingredients including radish, garlic, and red pepper powder (Cho et al., 1999). The growth patterns of lactic acid bacteria in kimchi and sauerkraut are similar, with the *Leuconostoc* bacterial genus dominating the initial lactic acid production phase during fermentation (Eom et al., 2008). During this stage, *Leuconostoc* produces metabolites such as dextran, lactate, acetate, alcohol, CO_2, and mannitol, all of which contribute to the taste of the fermented foods (Chyun and Rhee, 1976). The ingestion of a typical watery kimchi, dongchimi, which is usually made from radish, produces 3 distinct taste sensations: a sour taste due to organic acids, a carbonated taste from CO_2, and a sweet taste due to sugars and sugar alcohols.

The enzyme dextransucrase (EC 2.4.1.5), expressed by various species of the genus *Leuconostoc*, transfers the glucosyl moiety of sucrose to form dextran, an α-(1 → 6)-linked D-glucan. Dextransucrase also catalyzes the transfer of glucose from sucrose (the donor) to other carbohydrates (the acceptors) by cross-linking the α-(1 → 6)-glucosyl bond. Using maltose as the acceptor molecule, several isomaltooligosaccharides (IMOs) were produced in an experiment using *Leuconostoc mesenteroides* NRRL B-512F (Robyt and Eklund, 1983). IMO is a representative prebiotic and an α-(1 → 6)-linked glucooligosaccharide. IMO found in commercial products shows a degree of polymerization that ranges from 2 to 6 (Crittenden and Playne, 1996; Han et al., 2002). A prebiotic resists digestion in the upper gastrointestinal tract, but is selectively metabolized in the colon by beneficial microbes and consequently offers health benefits (Gibson et al., 2004). Olano-Martin et al. (2000) reported that oligodextran can be used by bifidobacteria and lactobacilli, and it was registered as a health-promoting prebiotic by the Korean Food and Drug Administration (http://www.foodnara.go.kr/hfoodi/).

In a previous study (Han et al., 2002), we applied the acceptor reaction of dextransucrase in kimchi by adding sucrose and maltose, and found that IMOs were synthesized during the lactate fermentation period as expected. The same reaction occurred in kefir-like milk fermentation when sucrose and maltose were added with a *Leuconostoc* starter (Seo et al., 2007). To enhance IMO production, we previously isolated psychrotrophic *Leuconostoc citreum* (*L. citreum*) KACC 91035 that has high dextransucrase activity (Eom et al., 2007). The current study

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used *L. citreum* KACC 91035 as a starter strain in the kimchi fermentation process to enhance the production of IMO during lactate fermentation.

2. Materials and methods

2.1. Materials, bacterial strains, and culture condition

Sucrose, maltose, NaCl, and panose were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *L. citreum* KACC 91035 used as the starter strain was cultured in MRS broth. A liquid medium to inoculate the kimchi was prepared by autoclaving and filtering the extracted cabbage. Cubic plastic jars with sealing lids were used as fermentation vessels. Radish, red pepper, green onion, and other kimchi ingredients were purchased from a local grocery store.

2.2. Preparation of kimchi (dongchimi-kimchi)

To prepare dongchimi-kimchi, a whole radish (800 g) was minced and mixed with salt (40 g), green onions (20 g), garlic (10 g), and ginger (3 g). Next, the kimchi samples were sorted according to sugar and starter addition: A, kimchi with no added sugar (blank); B, kimchi with 58 mM sucrose and 56 mM maltose; and C, kimchi with 58 mM sucrose, 56 mM maltose, and the starter (10^7 colony forming units (CFU)/mL). The jars were filled to 4 L with drinking water and tightly sealed with plastic lids. The temperature during the fermentation process was maintained at 10 °C for 7 d, after which the temperature was dropped to 4 °C for preservation.

2.3. Microbial and chemical analyses

The growth of lactic acid bacteria in the kimchi fermentation mix was measured in CFU/mL using the culture pouring method with Lactobacilli (LAB) MRS agar medium. The kimchi fermentation mix was diluted with 0.85% (w/v) physiological saline and poured on agar plates that were incubated at 28 °C for 48 h. The pH of the test solution was determined with a pH meter (IQ240; IQ Scientific Instruments, San Diego, CA, USA).

2.4. Sugar analysis

The sugars present in the kimchi fermentation mix were analyzed using a High Performance Anion Exchange Chromatography (HPAEC) system (Bio-LC ICS-3000, Dionex Corp., Sunnyvale, CA, USA) fit with a CarboPac PA1 column (0.2 × 25 cm, Dionex) and a pulsed amperometric detector (ED50, Dionex). For quantitative and qualitative analyses of peaks, we used the software Chromate Window v.3.0 (Interface Engineering Inc., Portland, OR, USA). In parallel, TLC analysis was used to monitor oligosaccharide synthesis. For the analysis of mono- or di-saccharides, TLC plates (Whatman K5 TLC plates, Merck, Darmstadt, Germany) were developed thrice with acetonitrile/distilled water (85:15, v/v); the separated sugars were detected by dipping the plates in ethanol containing α-naphthol (0.5%, w/v) and sulfuric acid (5%, v/v), followed by heating at 110 °C for 5 min (Lee et al., 2008).

2.5. PCR-DGGE analysis

The kimchi fermentation mix was grinded and filtered through sterilized cheese cloth twice. Genomic DNA from kimchi fermentation mix was extracted using the Genomic DNA Prep kit for bacteria (SolGent, Daejeon, Korea) according to manufacturer's instructions. The 16S rRNA gene V3 regions were amplified using the universal bacterial primers 338f and 518r. The sequences of the primers were as follows: 338f, 5′-ACTCCTACGGGAGGCAGCAG-3′ (*Escherichia coli* positions 338 to 357) and 518r, 5′-ATTACCGCGGCTGCTGG-3′. The forward primer, 338f, had a GC clamp. Amplified DNA sequences were resolved by electrophoresis at 60 °C, and to increase resolution, we used gels with a 30–60% urea: formamide denaturing gradient (100% corresponding to 7 M urea and 40% [wt vol^{-1}] formamide) increasing in the direction of electrophoresis. Samples were electrophoresed for 30 min at 20 V and for 16 h at 60 V, stained with EtBr for 1 h, and photographed under UV illumination. For microbial identification, gel bands were purified with the Gel & PCR Purification System (SolGent) and sequenced after PCR re-amplification (without the GC-clamp); sequences were compared to those in the GenBank database using the BLAST algorithm (National Center Biotechnology Information, MA, USA).

3. Results

3.1. The fermentation profile of kimchi

Three batches of dongchimi-kimchi were prepared: kimchi A, with no sugar; kimchi B, with sucrose and maltose; and kimchi C, with sucrose, maltose, and the starter. The batches were fermented at 10 °C for 7 d then stored at 4 °C thereafter (Fig. 1A); during this period, the biochemical changes in kimchi were monitored. The low temperature (10 °C) maintained during fermentation stage enables the *Leuconostoc* starter to become the dominant species and secrete dextransucrase. The initial pH in all 3 batches of kimchi was approximately 6.4, but after 1 d, the pH of kimchi C dropped rapidly to 4.2, a value lower than that in kimchi A and B; this is likely due to the increased growth of kimchi C as a result of starter addition. Over a 13-d monitoring period, the pH of kimchi A, B, and C decreased to 3.8, 3.7, and 3.7, respectively (Fig. 1B). Total LAB cell numbers of kimchi A and B increased slowly to approximately 10^8 CFU/mL after 7 d, whereas those numbers were reached in kimchi C after just 1 d (Fig. 1C). When PCR-DGGE analysis was used to monitor microbial dynamics during kimchi fermentation (Fig. 1D), *Pseudomonas* (bands *a* and *b*) and *Enterobacteriaceae* sp. (bands *c* and *f*) were initially found in kimchi B, but *Leuconostoc gasicomitatum* (band *e*) and *Lactococcus piscium* (band *d*) quickly became the dominant LAB species. In contrast, in kimchi C, *L. citreum* KACC 91035 (band *g*) was dominant throughout fermentation.

3.2. The effect of starter addition on IMO production

The transfer of glucose to an acceptor molecule, which is catalyzed by dextransucrase, was used for IMO synthesis in kimchi. Maltose was added as the acceptor molecule during kimchi preparation, and the changes in sugar concentration were analyzed by TLC and HPAEC (Fig. 2). As expected, dextransucrase catalyzed the transfer of glucose from sucrose to the maltose acceptor, producing panose, isomaltosyl maltose (IMM), and isomaltotriosyl maltose (IM3M). The results of TLC (Fig. 2A) and HPAEC (Fig. 2B) analyses of kimchi C showed that sucrose (58 mM) was rapidly consumed within 3 d, and nearly half the maltose (28 mM), which acted as an acceptor molecule was used up. Simultaneously, the IMOs were synthesized and fructose was released from sucrose.

The concentrations of oligosaccharides synthesized in kimchi B and C were also monitored (Fig. 3). IMOs were not synthesized in kimchi A to which no sugars were added (Fig. 3A). In contrast, with sucrose and maltose added in kimchi B, IMOs were immediately produced, and their concentrations peaked after 3 d and were maintained at that level for 13 d at 4 °C. Sucrose (58 mM) and maltose (56 mM) were converted into panose (50.49 mM), IMM (22.8 mM), and IM3M (1.98 mM). In kimchi C, which included the starter culture of *L. citreum* KACC 91035 in addition to sucrose and maltose, the production of IMO dramatically increased, with the total IMO concentration increasing to over 100 mM: panose (72.71 mM), IMM (32.53 mM), and IM3M (4.95 mM). As summarized in Table 1, in the non-starter-added kimchi B, the production rate and maximal concentration of IMOs were 15.05 mM/d and 75.27 mM, respectively, whereas in the starter-added kimchi C the rate and concentration were increased to 22.04 mM/d and 110.19 mM, respectively. After the IMOs had accumulated in kimchi, their concentrations decreased