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RESEARCH NOTE

Simple Synthesis of Isomaltooligosaccharides during *Sauerkraut* Fermentation by Addition of *Leuconostoc* Starter and Sugars

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Abstract Isomaltooligosaccharides (IMOs), prebiotic compounds stimulating the growth of intestinal bacteria, were synthesized in *sauerkraut* by inoculating psychrotrophic *Leuconostoc citreum* KACC 91035 strain with high dextransucrase activity. For the glucose transferring reaction of dextransucrase, sucrose and maltose were added in *sauerkraut* (29 and 28 mM, respectively, w/v) as glucosyl donor and acceptor molecule, respectively. For 12 days of fermentation at 10°C, IMOs were gradually produced by consuming sucrose and maltose, and the synthesis rate and maximal concentration of IMOs were 2.04 and 20.2 mM, respectively. This result demonstrates a simple method to manufacture a synbiotic *sauerkraut* product by adding probiotic lactic acid bacterium and sugars as ingredients.

Keywords: *Leuconostoc citreum*, dextransucrase, isomalto-oligosaccharide, prebiotics, *sauerkraut*

Introduction

Sauerkraut is a traditional German vegetable food which is currently consumed in many countries in the world (1). It is a product of lactic acidic fermentation spontaneously developed by lactic acid bacteria (LAB) under anaerobic conditions with the use of a certain amount of salt at optimal temperatures. It is known that *Leuconostoc* is a dominant genus leading the initial phase of lactic acid production during *sauerkraut* fermentation (2,3). During these stages, *Leuconostoc* spp. produce various metabolites such as lactate, acetate, alcohol, CO_2, and mannitol, all of which contribute to the flavor of the fermented foods (4). In addition, *Leuconostoc* spp. produce dextran polymer by secreting dextransucrase. Dextransucrase catalyzes the transglycosylation reaction to form α-(16)-glucan from sucrose (5). Specifically, this enzyme produces isomalto-oligosaccharides (IMOs) in the presence of maltose by transferring glucose moiety from sucrose to maltose (6). IMOs contain panose (α-D-Glc-(16)-α-D-Glc-(14)-D-Glc) as a major component (7) and they provide bifidogenic effect on the colonic microbiota of human (7-9).

In previous studies (10), we applied the acceptor reaction of dextransucrase in kimchi by adding sucrose and maltose, and found that IMOs were synthesized successfully. To enhance IMOs production, we previously isolated psychrotrophic *Leuconostoc citreum* KACC 91035 that has high dextransucrase activity (11), and used the strain as a starter culture for the kimchi fermentation process (12). In this study, synthesis of IMOs during lactate fermentation of *sauerkraut* was attempted by adding *L. citreum* KACC 91035 strain as a starter strain and sugars (sucrose and maltose) as substrates.

Materials and Methods

Materials, bacterial strains, and culture condition Sucrose, maltose, NaCl, and standard chemicals were

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purchased from Sigma Chemical Co. (St. Louis, MO, USA). *L. citreum* KACC 91035 used as the starter strain was cultured in MRS broth. A liquid medium to inoculate the *sauerkraut* was prepared by autoclaving and filtering the extracted cabbage. Cubic plastic jars with sealing lids were used as fermentation vessels. Cabbage was purchased from a local grocery store.

Preparation of *sauerkraut* To prepare *sauerkraut*, fresh cabbage (5 kg) was trimmed of outer leaves and shredded to 1 mm thick with a food slicer. The shredded cabbage was mixed with salt to obtain a final concentration of 2% (w/w) NaCl in 10 L glass jars, covered with flexible plastic film, and weighted down with heavy stone on top of the film (13). For transglycosylation reaction *L. citreum* KACC 91035 was inoculated as a starter (10^7 colony forming units (CFU)/mL) and sucrose (29 mM) and matose (28 mM) as glucosyl donor and acceptor compounds, respectively. 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.

Microbial and chemical analyses The growth of LAB in the *sauerkraut* was measured in CFU/mL using the pour plate method on MRS agar medium. The liquid 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). The sugars present in the *sauerkraut* mix were analyzed using a High Performance Anion Exchange Chromatography (HPAEC) system (Bio-LC ICS-3000; Dionex Co., Sunnyvale, CA, USA) with a CarboPac PA1 column (0.2×25 cm; Dionex Co.) and a pulsed amperometric detector (ED50; Dionex Co.). For quantitative and qualitative analyses of peaks, the software Chromate Window v.3.0 (Interface Engineering Inc., Portland, OR, USA) was used.

Results and Discussion

The fermentation profile of *sauerkraut* *Sauerkrauts* were prepared in 3 batches: *sauerkraut* A, with no sugar; *sauerkraut* B, with sucrose and maltose; and *sauerkraut* C, with sucrose, maltose, and the starter. The batches were fermented at 10°C for 17 days and the biochemical changes in *sauerkraut* were monitored. The initial pH in all 3 batches of *sauerkraut* was approximately 5.7, but after 3 days, the pH of *sauerkraut* C dropped rapidly to 4.0 due to the increased growth of *sauerkraut* C as a result of starter addition (Fig. 1A). Figure 1B shows the changes in the viable counts of LAB during the *sauerkraut* fermentation. The total LAB counts for *sauerkraut* C (initial counts; 10^7 CFU/mL) increased for 3 days, and then maintained until the end of the storage period (17 days), whereas total LAB populations in *sauerkraut* A and B increased slowly to approximately 10^8 CFU/mL after 12 days.

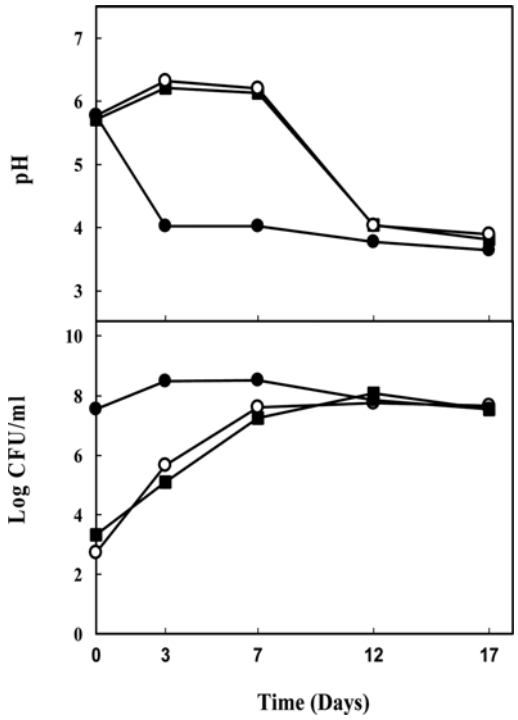

Fig. 1. Profiles of pH change (A), and cell growth in the microbial populations (B) during *sauerkraut* fermentation. ■, *sauerkraut* with no sugar; ○, *sauerkraut* with 29 mM sucrose and 28 mM maltose; ●, *sauerkraut* with 29 mM sucrose and 28 mM maltose, and starter (10^7 CFU/mL).

IMOs production and the effect of starter inoculation Sucrose and maltose were added to the *sauerkraut* with starter, and the changes in the sugar concentration in the *sauerkraut* during the fermentation were then analyzed by 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). In all cases, sucrose (29 mM) was rapidly consumed within 7 days, whereas only about half of the maltose (14 mM) was used as acceptor molecules (Fig. 3). In *sauerkraut* B with sucrose and maltose, IMOs were slowly produced and their concentrations peaked after 12 days and were decreased at that level for 17 days at 10°C (Fig. 3B). As expected, sucrose (29 mM) and maltose (28 mM) were converted into panose (8.63 mM), IMM (6.56 mM), and IM3M (1.74 mM). In constant, IMOs were not synthesized in *sauerkraut* A to which no sugars were added (Fig. 3A). This result shows that IMOs can be produced in *sauerkraut* by simple addition of sucrose and maltose before preparation of foods. Notably, in *sauerkraut* C including the starter culture as well as

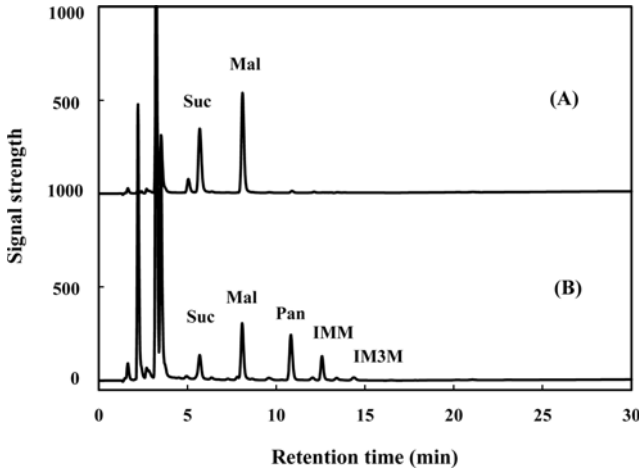

Fig. 2. Time course of changes in individual sugar levels during *sauerkraut* (Sample C) fermentation with 29 mM sucrose, 28 mM maltose, and starter. The sugar contents were analyzed using HPAEC at 0 day (A), 7 days (B). Suc, sucrose; Mal, maltose; Pan, panose; Std, standard molecules. IMM, isomaltosyl maltose; IM3M, isomaltotriosyl maltose.

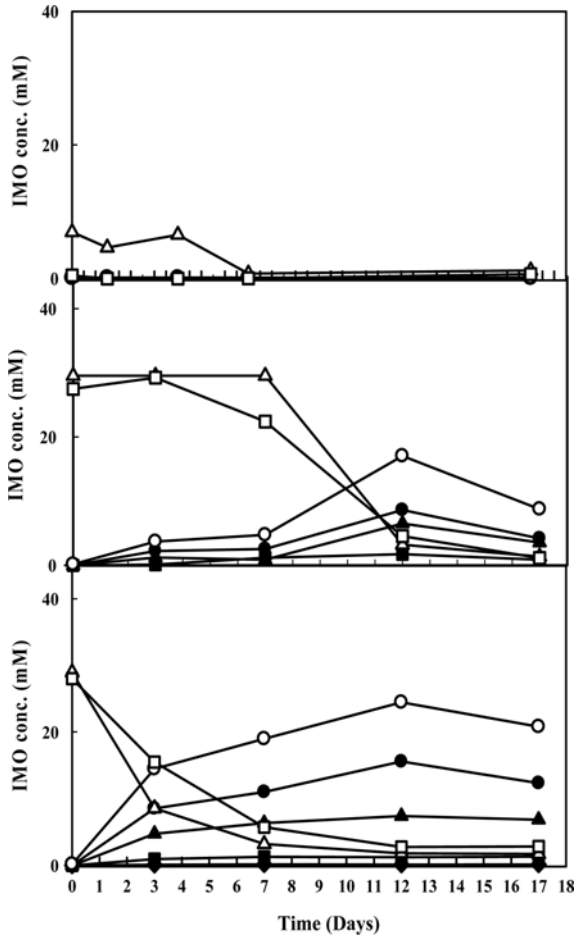

Fig. 3. Profile of sugar changes in *sauerkraut* (A), in *sauerkraut* with 29 mM sucrose and 28 mM maltose (B), and in *sauerkraut* with 29 mM sucrose, 28 mM maltose, and starter (C). ●, panose; ▲, isomaltosyl maltose; ■, isomaltotriosyl maltose; ◆, isomaltotetraosyl maltose; ○, total IMOs; △, sucrose; □, maltose. Data are from 3 independent experiments.

sugars, IMOs concentrations highly increased after 12 days (Fig. 3C); panose (11.59 mM), IMM (7.40 mM), IM3M (1.22 mM), and IM4M (0.11 mM). This result demonstrates that the production yield of IMOs can be significantly improved by addition of psychrotrophic *L. citreum* KACC 91035 showing high dextransucrase activity. As summarized in Table 1, in the non-starter-added *sauerkraut* B, the production rate and maximal concentration of IMOs were 1.41 mM/day and 16.88 mM, respectively, whereas in the starter-added *sauerkraut* C the rate and concentration were increased up to 2.04 mM/day and 20.21 mM, respectively.

Sauerkraut contains several LAB species, including *L. mesenteroides*, *L. citreum, L. argentinum, Lactobacillus plantarum*, *Lb. brevis, Lb. rhamnosus, Lb. coryniformis,* and *Weissella* spp. showing *Leuconostoc* spp. are predominant (2,14-17). *L. citreum* gives a unique taste in *sauerkraut* by providing with appropriate condition for lactate fermentation. The bacterium grows rapidly during the initial stage of fermentation and scavenges oxygen from the liquid to create anaerobic conditions for lactic acid fermentation. The bacterium also produces lactic acid and CO_2 through the hetero-lactic acid fermentation pathway, giving a sour and carbonated taste to *sauerkraut*. Particularly in *sauerkraut* preparation, the literature review reveals that sugar addition has not been conducted, probably due to the higher production of organic acids and dextran polymers. However, in this study, most of sugars added were converted into IMOs after fermentation and the polymer synthesis was avoided. In addition, the resulting fructose moieties from sucrose can be converted into mannitol providing sweet taste to the fermented foods.

In conclusion, the glucose-transferring dextransucrase action of *L. critreum* dextransucrase usage enables the production of beneficial oligosaccharides in *sauerkraut*. In

Table 1. Comparison of IMOs concentrations in *sauerkraut* after 12 days of the dextransucrase reaction

Types of *sauerkraut*	Pan[1)] (mM)	IMM (mM)	IM3M (mM)	Total IMOs (mM)	Production rate (mM/day)
No sugar (A)	0	0	0	0	0
Suc+Mal (B)	8.63	6.51	1.74	16.88	1.41
Suc+Mal+Starter (C)	11.59	7.40	1.22	20.21	2.04

[1)]Pan, panose; IMM, isomaltosyl maltose; IM3M, isomaltotriosyl maltose; Total IMOs, Total isomaltooligosaccharides

proper condition unfavorable polymer synthesis is avoided and the level of sweetness is maintained. The application of this method will allow the biocatalytic synthesis of IMOs during lactate fermentation for the future development of new functional lactate foods.

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Disclosure The authors declare no conflict of interest.

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