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Research Note

Comparison of Bifidogenic Growth Stimulation Activities of Fermented Whey Prototypes

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ABSTRACT: Fermented whey solution presenting bifidogenic growth stimulation (BGS) activity was processed as prototypes such as sterilized fermented whey (SFW), spray-dried fermented whey (SDFW), and freeze-dried fermented whey (FDFW) and their BGS activities were compared. In optical density (OD_{600}) test, the BGS activity of three prototypes, which showed similar activities, were significantly different with non-fermented whey solution adjusted to pH 4.5 as a control ($P<0.05$). In viable cell count test, SDFW had the most positive influence than other prototypes on the BGS activity even though the difference was not significant. However, the activities of all prototypes were significantly different than the negative control (no addition). These results indicate that the processed prototypes of fermented whey solution show BGS activities and might be commercialized, with further evidences, in animal or human studies.

Keywords: fermented whey, bifidogenic growth stimulation, *Lactobacillus casei*, *Leuconostoc mesenteroides*

INTRODUCTION

Bifidobacteria are well-known in academia and dairy industry and have positively influenced human intestinal physiology (1,2). Particularly, bifidobacteria modulate intestinal microbiota and eradicate harmful pathogenic bacteria. Recently, a research paper presented particular bifidobacterial strains that can effectively protect enteropathogenic *Escherichia coli* O157:H7 by producing acetate, which shows anti-inflammatory and anti-apoptotic effects, and prevents the translocation of Stx2 toxin to the blood (3). Some bifidobacterial strains also present bile salt hydrolase activity, which cleaves conjugated bile salts resulting in lowering blood cholesterol level (4). A bifidobacterial cell extract has been shown to inhibit growth of colon cell lines such as Caco-2, HT-29, and SW480 and stimulates murine macrophage RAW 264.7 cells (5). Besides the above mentioned activities, bifidobacteria have several functions in human intestines including prevention of diarrhea, improvement of atopic eczema, and production of beneficial metabolites such as vitamins (6,7). Due to these functional effects, researchers have paid much attention to the bacteria. Needless to say, maintenance of the bacteria in human intestines is very important in controlling our intestinal physiology. To meet this demand, many probiotic agents such as oligosaccharides have been developed (8,9). In our previous study, we also tried to choose lactic acid bacteria which present bifidogenic growth stimulation (BGS) activity via whey fermentation (10-12). From the study, two lactic acid bacteria, *Leuconostoc mesenteroides* CJNU 0147 and *Lactobacillus casei* CJNU 0588, were finally chosen for BGS activity. In this study, commercial potential of processed fermented whey prototypes, prepared with mixed culture of *Leu. mesenteroides* CJNU 0147 and *L. casei* CJNU 0588 strains, such as sterilized fermented whey (SFW), spray-dried fermented whey (SDFW), and freeze-dried fermented whey (FDFW) were evaluated.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bifidobacterium lactis BL 750 (Culture Systems Inc., Mishawaka, IN, USA) and *Bifidobacterium longum* FI10564 (13) were cultured in RCM (Reinforced Clostridial Medium, Becton, Dickinson and Co., Franklin Lakes, NJ, USA) broth at 37°C in an anaerobic jar (Oxoid Ltd., Cambridge, UK) with BD GasPak™ EZ Anaerobic Container System Sachets (Becton, Dickinson and Co.) for BGS activity tests.

Production of fermented whey prototypes

As mentioned in our previous paper (10), 0.5% (w/v) of

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both freeze-dried cells (*Leu. mesenteroides* CJNU 0147, 4.68×10^9 CFU/g; *L. casei* CJNU 0588, 3.44×10^9 CFU/g) was inoculated in 75 L of sterilized whey broth (10%, w/v) and incubated for 48 h under optimal conditions, i.e. mixed culture of two strains for starter, 20°C for culture temperature, and non-air supply into culture medium. The fermented whey was prepared as three prototypes: sterilized fermented whey (SFW; treated at 60°C for 30 min), spray-dried fermented whey (SDFW; treated at 97°C with a drying capacity of 1 kg/h), and freeze-dried fermented whey (FDFW) (Fig. 1). All the samples were stored at 4°C until use.

BGS activity test

Prepared fermented whey prototypes were compared for BGS activity using two bifidobacterial strains, *B. lactis* BL 750 and *B. longum* FI10564. Briefly SFW, SDFW, and FDFW samples, where the last two samples were suspended with distilled water to a final concentration of 10% (w/v), were centrifuged at 6,000 rpm for 10 min and the supernatants were filtered with a syringe filter (0.45 μm, Merck Millipore, Billerica, MA, USA). One hundred μL of each filtrate was added into 5 mL of RCM broth which was inoculated with *B. lactis* BL 750 or *B. longum* FI10564 and anaerobically incubated at 37°C for 14 h and their optical density at 600 nm was measured and compared with controls. For viable cell count test, 50 μL of each prototype sample without filtration was added into 5 mL of RCM broth which was inoculated with *B. lactis* BL 750 or *B. longum* FI10564 and anaerobically incubated at 37°C. At 0, 6, 9, 12, 18, and 24 h, the viable cell counts of bifidobacteria were checked on RCM agar plates with colony PCR (primers Bif164: 5'-GGGTGGTAATGCCGGATG-3' and Bif664: 5'-CCACCGTTACACCGGGAA-3') for confirmation.

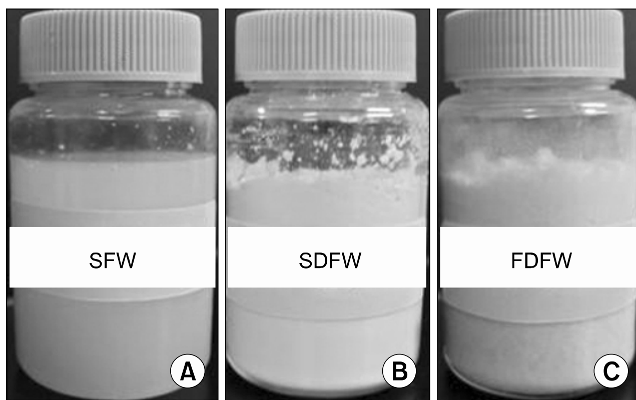


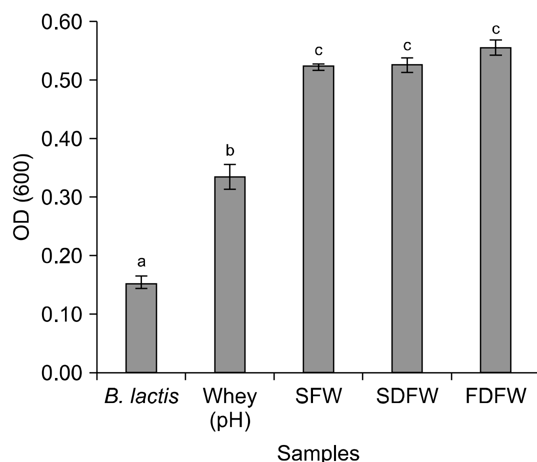
Fig. 1. Prototypes of fermented whey solution. (A) Sterilized fermented whey (SFW), (B) Spray-dried fermented whey (SDFW), (C) Freeze-dried fermented whey (FDFW).

Statistical analysis

All experiments in this study were done in triplicate and data are represented as mean or mean±standard deviation (SD). A statistical software (SPSS v. 12.0; SPSS Co., Chicago, IL, USA) was used for Duncan's multiple range tests for determining significance of difference ($P<0.05$).

RESULTS AND DISCUSSION

Fermented whey prototypes SFW, SDFW, and FDFW were compared for BGS activities by using optical density (OD_{600}) and viable cell counts. In optical density test (Fig. 2), after 14 h the OD_{600} value of control, where only *B. lactis* BL 750 strain was inoculated, was 0.154 and that of another control, where non-fermented whey solution adjusted to pH 4.5 with lactic acid was added to the inoculated RCM broth, was 0.335, whereas those of SFW, SDFW, and FDFW were 0.523, 0.525, and 0.555, respectively; these values were significantly different than



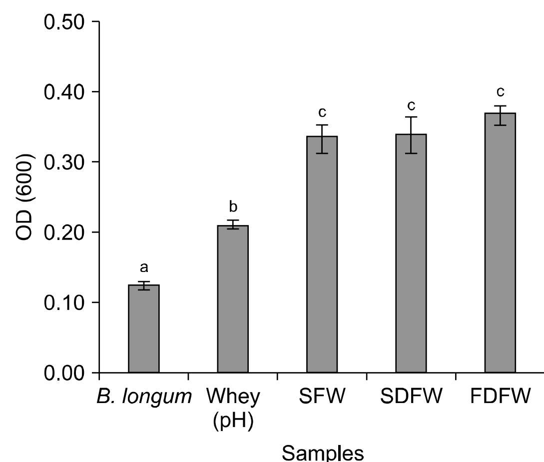


Fig. 2. Bifidogenic growth stimulation activity test with optical density at 600 nm in RCM broth at 37°C for 14 h. *B. lactis* or *B. longum*, only bifidobacterial cells were inoculated; Whey (pH), bifidobacterial cells plus non-fermented whey solution adjusted to pH 4.5 with lactic acid; SFW, plus sterilized fermented whey; SDFW, plus spray-dried fermented whey; FDFW, plus freeze-dried fermented whey.

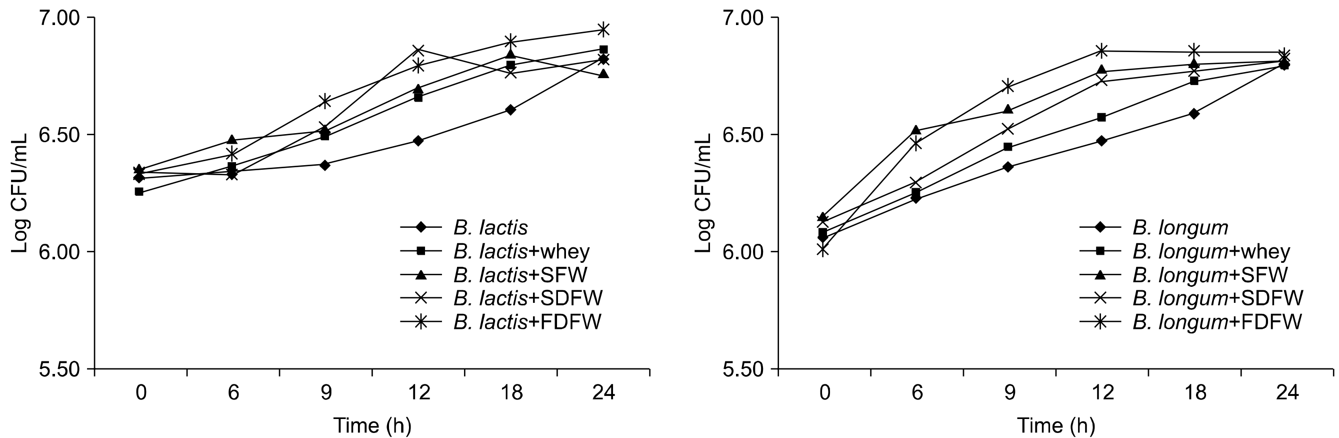


Fig. 3. Bifidogenic growth stimulation activity test with viable cell counts during cultivation in RCM broth at 37°C. *B. lactis* or *B. longum*, only bifidobacterial cells were inoculated; Whey, bifidobacterial cells plus non-fermented whey solution adjusted to pH 4.5 with lactic acid; SFW, plus sterilized fermented whey; SDFW, plus spray-dried fermented whey; FDFW, plus freeze-dried fermented whey.

controls. In the same experiment for *B. longum* FI10564, similar results were obtained. The OD_{600} value of a control, where only *B. longum* FI10564 strain was inoculated, was 0.125 and that of another control, where the non-fermented whey solution was added, was 0.210, whereas those of SFW, SDFW, and FDFW were 0.334, 0.340, and 0.368, respectively. Unexpectedly, the values of optical density for 3 fermented whey prototypes samples were not significantly different even though they were subjected to different processes. These results indicate that the bifidogenic growth stimulator in the fermented whey solution was not influenced by the processes including sterilization, spray-drying, and freeze-drying. In viable cell count test (Fig. 3), all the prototypes presented BGS activity. Unlike the results of optical density test, those of viable cell counts of the prototypes were slightly different. Broadly, FDFW showed most effective BGS activity for *B. lactis* BL 750 and *B. longum* FI10564 strain during cultivation until 18 h but there was no significant difference between SFW and SDFW for both strains. Unexpectedly non-fermented whey solution showed similar BGS activity to SFW for *B. lactis* BL 750 strain. Conclusively, all the fermented whey prototypes presented BGS activities and particularly FDFW is a more desirable prototype even though the BGS activity was not significantly different with other prototypes since it contains live probiotic lactic acid bacteria *Leu. mesenteroides* CJNU 0147 and *L. casei* CJNU 0588.

Presently, intestinal commensal bacteria are recognized as an important player for human physiology and their protective influence against gut dysbiosis and intestinal diseases such as diarrhea, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), enteritis, etc. As commensal bacteria, bifidobacteria play beneficial roles in human intestines and therefore are used as an indicator for healthy intestinal state. Due to their beneficial effects on human health, several bifidobacterial strains have already been commercialized and many other strains are under development for commercial purposes (14). The fermented whey prototypes developed in this study can support survivability and functions of these kinds of bifidobacteria in human intestines and can be commercialized as functional foods or materials. In the future, the prototypes should be applied to animal tests for proving their probiotic effects and more reasonable evidences as bifidogenic growth stimulator.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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