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RESEARCH NOTE

Optimal Production of Fermented Whey Presenting Bifidogenic Growth Stimulator Activity

Jong-Kwang Lee, Ji-Eun Eom, Hyun-Soo Shin, Weon-Sun Shin, and Gi-Seong Moon

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Abstract Various culture conditions for the production of fermented whey presenting bifidogenic growth stimulator (BGS) activity were evaluated using *Leuconostoc mesenteroides* CJNU 0147 and *Lactobacillus casei* CJNU 0588. The BGS activity of fermented whey produced with mixed culture of *Leu. mesenteroides* CJNU 0147 and *L. casei* CJNU 0588 was higher than those of single cultures. The optimal temperature for the production of the fermented whey was 20°C. The anaerobic culture conditions via nitrogen gas supply had no influence on the BGS activity of fermented whey. The BGS activity of the heat-treated fermented whey samples was slightly decreased by 7.63, 11.66, and 15.12% at 80, 100, and 121°C, respectively for 15 min. Pilot-scale (75 L) fermented whey was produced using the 2 freeze-dried cell powders of CJNU 0147 and CJNU 0588 and spray-dried. The spray-dried fermented whey presented BGS activity, indicating it can be used as a functional food material.

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

Jong-Kwang Lee, Ji-Eun Eom, Gi-Seong Moon (✉)
Department of Biotechnology, Chungju National University, Jeungpyeong, Chungbuk 368-701, Korea
Tel: +82-43-820-5251; Fax: +82-43-820-5272
E-mail: gsmoon@cjnu.ac.kr

Jong-Kwang Lee
JKNUTRA Co., Seoul 138-805, Korea

Hyun-Soo Shin
R & D Center, Namyang Dairy Products Co., Ltd., Gongju, Chungnam 314-914, Korea

Weon-Sun Shin
Department of Food and Nutrition, Hanyang University, Seoul 133-791, Korea

Introduction

Bifidobacteria are recognized as lactic acid bacteria (LAB) even though they are not phylogenetically included in the LAB group (1). They are Gram-positive bacteria and are strictly anaerobic, such that they cannot survive in normal environments exposed to oxygen. Due to this reason, they normally reside in the gastrointestinal (GI) tracts of humans and animals (2).

The bacteria are one of the most important microorganisms for the balance of human intestinal microbiota. They also have various beneficial effects on human health such as inhibition of harmful bacteria (3), protection against diarrhea (4), alleviation of constipation (5), stimulation of immune response (6), prevention of tumor cell growth (7), etc.

For these reasons, bifidobacteria in our GI tract is very important, and the research and developments in dairy and functional food industries have focused on the subject. In general, bifidobacteria have been supplied to human intestines as probiotics in dairy products or functional food products; however, it is not clear that they can survive and colonize in the intestines. So, it might be desirable to intake prebiotics such as fructo-oligosaccharide (FOS) (8), galacto-oligosaccharide (GOS) (9), inulin (10), and raffinose (11) or bifidogenic growth stimulator (BGS) such as 1,4-dihydroxy-2-naphthoic acid (DHNA) and 2-amino-3-carboxy-1,4-naphthoquinone (ACNQ), which can selectively stimulate the growth of bifidobacteria colonized in human intestines, resulting in the improvement of the intestinal environment (12-14).

Cheese whey is recognized as a good medium for the fermentation of LAB since it contains organic compounds including lactose as a carbon source (15). It also contains functional proteins such as α-lactalbumin, β-lactoglobulin, immunoglobulin, lactoferrin, and glycomacropeptides (GMP)

(16,17). Despite its beneficial properties, the price of whey is still comparatively cheap, making it useful as a broad fermentation medium for the production of commercial metabolites from microorganisms.

In this study, we tried to determine the optimal conditions for the production of fermented whey presenting BGS activity using 2 LAB isolates, *Leuconostoc mesenteroides* CJNU 0147 and *Lactobacillus casei* CJNU 0588, which were previously reported as strains producing unknown BGS (18,19), and produce fermented whey in pilot-scale.

Materials and Methods

Bacterial strains and culture conditions *Leuconostoc mesenteroides* CJNU 0147 and *Lactobacillus casei* CJNU 0588 strains were activated in MRS broth (BD, Sparks, MD, USA) at 37°C without shaking and sub-cultured in 10%(w/v) whey medium (Samik Dairy & Food Co., Seoul, Korea) for the production of fermented whey. *Bifidobacterium longum* FI10564 and *Bifidobacterium lactis* BL 750 (Culture Systems Inc., Mishawaka, IN, USA) were activated in reinforced clostridial medium (RCM, BD) broth at 37°C in an anaerobic jar (Oxoid, Cambridge, UK) for BGS activity tests.

Optimal conditions for production of fermented whey presenting BGS activity To investigate the optimal conditions for the production of fermented whey presenting BGS activity, single/mixed culture, temperature, and aerobic/anaerobic conditions were evaluated. Briefly, at first, the optimal conditions for the single and mixed cultures were determined, the optimal temperature was evaluated, and finally the effect of oxygen was investigated. For the tests, 1.5 L of 10%(w/v) whey medium in a 3-L jar fermenter (Fermentec Co., Cheongwon, Korea) was used. In the case of single culture, CJNU 0147 (2.69×10^9 CFU/mL) and CJNU 0588 (7.58×10^9 CFU/mL) strains were inoculated into the medium at a final concentration of 1%. In the case of mixed culture, both strains were inoculated simultaneously into the medium at a final concentration of 0.5%. To investigate the optimal temperature for the production, the fermented whey were produced at 20, 25, and 30°C. To test the air conditions of the culture for BGS activity, aerobic conditions with atmosphere and anaerobic conditions with nitrogen gas were applied to the jar fermenter. During fermentation under both conditions, sampling was performed at 0, 12, 24, 36, and 48 h, and viable cell counts expressed as colony forming unit (CFU)/mL were evaluated. The BGS activity was tested using 24 and 48 h samples. pH values were automatically measured and displayed by the jar fermenter.

BGS activity For the test of BGS activity of the fermented whey samples, 2 bifidobacterial strains, *B. longum* FI10564 and *B. lactis* BL 750, were used. Briefly, fermented whey was centrifuged at 6,000×*g* for 10 min. The resulting supernatant was filtered with a syringe filter (0.45-μm, Millipore Inc., Billerica, MA, USA), and the filtrate was used as the test sample. One-hundred μL of the filtrate was added to RCM broth (Difco), and a bifidobacterial strain was inoculated in the broth at 2%(v/v) concentration, after which the cells were incubated at 37°C for 10 h in an anaerobic jar supplemented with a GasPak EZ anaerobe container system (BD Inc., Sparks, MD, USA). The optical density at 600 nm (OD_{600}) was checked.

Heat stability of optimally produced fermented whey To evaluate food process applicability, heat stability of the fermented whey was tested. Optimally produced fermented whey was centrifuged and filtered as mentioned above. The sample was heat-treated at 80, 100, and 121°C for 15 min, and BGS activities were tested and compared with those of the untreated sample. Relative activity was calculated as a percentage of the BGS activity of the untreated sample.

Preparation of freeze-dried cells For the pilot-scale whey fermentation, *Leu. mesenteroides* CJNU 0147 and *L. casei* CJNU 0588 strains were freeze-dried. Briefly, each strain was inoculated in 30 L of skim milk, treated with a protease at 1%(v/v) concentration in a fermenter (Kobiotech Co., Incheon, Korea), and cultured at 30°C for 48 h. The culture was freeze-dried and stored at 4°C until use.

Pilot-scale production of fermented whey Exactly 0.5%(w/v) of both freeze-dried cells (*Leu. mesenteroides* CJNU 0147; 4.68×10^9, *L. casei* CJNU 0588; 3.44×10^9 CFU/g) was inoculated in 75 L of whey medium (10%, w/v), sterilized at 60°C for 30 min, and cultured for 48 h under optimal conditions as previously investigated. The fermented whey was concentrated, spray-dried, and stored at 4°C until use. The BGS activity of the spray-dried fermented whey was tested with *B. lactis* BL 750 strain according to the previously mentioned method.

Statistical analysis All experimental data are presented as mean±standard deviation (SD) of triplicate measurements. SPSS v. 12.0 (Statistical Package for Social Science Software: SPSS Co., Chicago, IL, USA) was used to perform Duncan's multiple range tests for determining significance of difference at $p<0.05$.

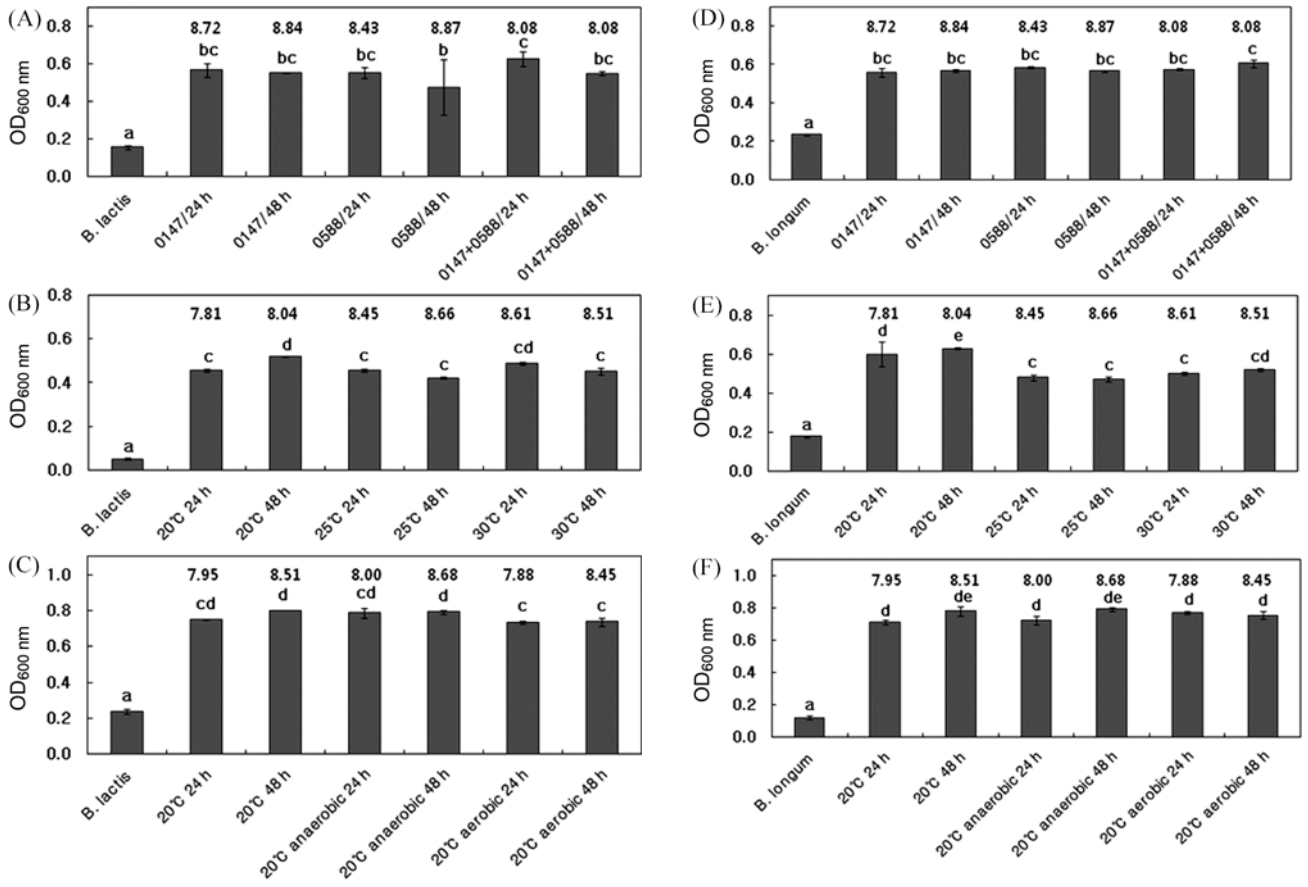

Fig. 1. BGS activity of fermented whey produced under various culture conditions. A, D: single or mixed culture; B, E: temperature; C, F: air condition. *B. lactis* BL 750 strain was used as a target bacterium in A, B, and C, and *B. longum* FI10564 strain was used in D, E, and F. For BGS activity test, 2 fermentation samples (24, 48 h) produced under each condition were used and compared with negative controls, where only *B. lactis* or *B. longum* strain was inoculated without adding the fermented whey samples. Figures on the bars, mean viable cell counts (log CFU/mL) of fermented whey samples produced with each condition; Values are mean±SD (n=3); Means having different letters are significantly different by Duncan's multiple range tests (p<0.05).

Results and Discussion

Optimal conditions for production of fermented whey presenting BGS activity In the single culture of *Leu. mesenteroides* CJNU 0147, the pH value decreased to 5.2 after 48 h, and the viable cell count reached 8.8 log CFU/mL. In the *L. casei* CJNU 0588 culture, the pH value decreased to 4.9, and the viable cell count reached 8.9 log CFU/mL. In the mixed culture of *Leu. mesenteroides* CJNU 0147 and *L. casei* CJNU 0588, the pH value decreased to 4.7, and the viable cell count reached 8.1 log CFU/mL. The viable cell counts and pH values of the single cultures were higher than those of the mixed culture. Normally, the decrease of pH value is proportional to the increase of viable cell count of LAB, since they use sugar as a carbon source to produce lactic acid. However, the theory is not applicable to our experimental results. Additionally, the BGS activity of the fermented whey from the mixed culture was slightly higher than those of the single cultures (Fig. 1A, 1D), indicating that production of BGS was increased by cellular competition. Therefore, the mixed culture was concluded to be the best for the production of fermented whey presenting BGS activity. Next, the optimal temperature was evaluated using the mixed culture. At 20ºC, the pH value decreased to 5.7, and the viable cell count reached 8.0 log CFU/mL after 48 h. At 25ºC, the pH value decreased to 5.3, and the viable cell count reached 8.7 log CFU/mL after 48 h. At 30ºC, the pH value decreased to 4.7, and the viable cell count reached 8.5 log CFU/mL after 48 h. As expected, the cell growth was fastest at 30ºC, as the highest viable cell count actually reached 9.0 log CFU/mL after 36 h. However, the BGS activity of the fermented whey did not correlate with cell growth, as the BGS activities for both bifidobacterial strains were highest at 20ºC (Fig. 1B, 1E). This result supports that BGS production from the mixed culture could be increased at hard condition for their growths. Therefore, 20ºC was chosen as the optimal temperature for the production of fermented whey. Finally, the optimal air conditions for BGS production were tested under previously

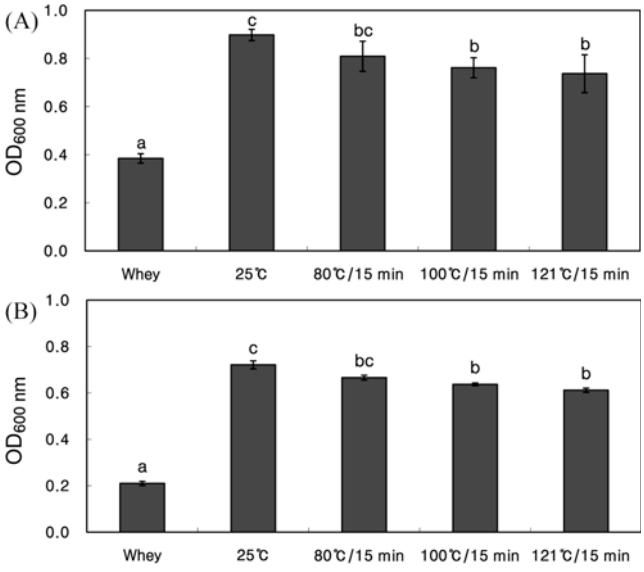

Fig. 2. Heat stability of the fermented whey. Target bacterium: *B. lactis* BL 750 strain (A), *B. longum* FI10564 strain (B); Whey medium, negative control. Values are mean±SD (n=3); Means having different letters are significantly different by Duncan's multiple range tests (p<0.05).

optimized conditions, such as mixed culture and 20°C. Under aerobic conditions with atmosphere, the pH value decreased to 5.8, and the viable cell count reached 8.4 log CFU/mL after 48 h. On the other hand, the pH value decreased to 5.7, and the viable cell count reached 8.7 log CFU/mL after 48 h under anaerobic conditions with nitrogen gas. The BGS activities of the fermented whey samples prepared under anaerobic conditions were slightly higher than those of the fermented whey samples under aerobic conditions; however, there was no difference between anaerobic condition and a condition without air supply (Fig. 1C, 1F). From the results, the condition without air supply was chosen for optimal production of fermented whey presenting BGS activity.

Heat stability of optimally produced fermented whey To evaluate food process applicability, the heat stability of optimally produced fermented whey was tested. When the BGS activity of fermented whey at 25°C was considered as 100%, decreases in activity were 9.92, 15.15, and 17.93% for *B. lactis* BL 750 strain and 7.63, 11.66, and 15.12% for *B. longum* FI10564 at 80, 100, and 121°C for 15 min, respectively (Fig. 2). Though the BGS activity slightly decreased at high temperatures, the rate of reduction was not significant. Therefore, the fermented whey could be applied to various food processes with mild heat treatment.

Pilot-scale production of fermented whey Pilot-scale (75 L) production of fermented whey was carried out under optimal conditions and spray-dried (Fig. 3A). The

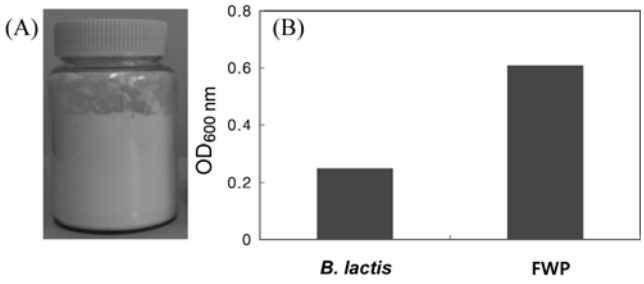

Fig. 3. Spray-dried fermented whey powder (A) and its BGS activity (B). *B. lactis* BL 750 strain was used as a target bacterium for BGS activity test. *B. lactis*: only *B. lactis* BL 750 strain was inoculated in RCM broth; FWP (fermented whey powder): *B. lactis* BL 750 strain was inoculated in RCM broth with fermented whey powder.

fermented whey powder still showed BGS activity for *B. lactis* BL 750 strain, and the optical density value at 600 nm was almost 2.4 times that of the negative control where the powder was not added. This result indicates that the spray-dried fermented whey powder could be used as a functional ingredient in various food products.

These days, intestinal microorganisms have gained interest based on their positive or negative roles in human health and physiology. As bifidobacteria are recognized as one of the most positive bacteria for human health, many researchers have focused on the improvement of the bacterial content in human intestines using prebiotics. This research area is still very attractive and novel agents having prebiotic effects have been identified. In this study, we tried to produce fermented whey powder presenting BGS activity in pilot-scale for the purpose of commercializing it in the future.

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