J Food Sci Nutr
Vol 16, p 386~389 (2011)
http://dx.doi.org/10.3746/jfn.2011.16.4.386

J Food Science and Nutrition

Leuconostoc mesenteroides CJNU 0147 and *Lactobacillus casei* CJNU 0588 Improve Growth of a *Bifidobacterium lactis* Strain in Co-cultures

- Research Note -

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Abstract

Previous studies have confirmed that fermented whey produced by *Leuconostoc mesenteroides* CJNU 0147 or *Lactobacillus casei* CJNU 0588 display bifidogenic growth stimulator (BGS) activity. The present study sought to determine if the strain itself can improve the growth of bifidobacteria in co-cultures. In reinforced clostridial medium (RCM), both strains stimulated the growth of a *Bifidobacterium lactis* strain during the exponential phase and also stimulated the growth during almost all growth phases in whey broth. Fermented whey containing viable *Leu. mesenteroides* CJNU 0147 and *L. casei* CJNU 0588 cells maintained viability of the *B. lactis* strain stored at 10°C in MRS broth. Viable cell count of the *B. lactis* strain without the fermented whey was decreased to 5.6 log cfu/mL after 15 days, whereas that of the strain with the fermented whey was slightly increased to 7.1 log cfu/mL as compared with initial viable cell count of 6.9 log cfu/mL.

Key words: *Leuconostoc mesenteroides* CJNU 0147, *Lactobacillus casei* CJNU 0588, bifidogenic growth stimulator, co-culture

INTRODUCTION

Bifidobacteria are one of the most widely-studied microorganisms, given their recognition as beneficial bacteria for human health and their exploitation as probiotics (1). Prebiotics, which can selectively stimulate beneficial bacteria such as bifidobacteria and lactobacilli in human intestines, have also been rigorously investigated (2). Prebiotics include non-digestible fibers such as inulin, fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), and different types of bifidogenic growth stimulators (BGSs) such as 2-amino-3-carboxy-1,4-naphthoquinone (ACNQ) and 1,4-dihydroxy-2-naphthoic acid (DHNA) (3). ACNQ is an electron acceptor for conversion of NAD^+ to NADH in bifidobacteria. DHNA is a precursor of vitamin K_2 (menaquinone), which is an electron carrier in the anaerobic respiratory chain of bacteria.

In our previous study, we tried to isolate DHNA-producing lactic acid bacteria via whey fermentation but could not identify any target bacteria, although two strains, *Leuconostoc mesenteroides* CJNU 0147 and *Lactobacillus casei* CJNU 0588, did present BGS activity (4,5). Presently, we don't know how these cells have influenced the growth of bifidobacteria. For example, *Leu. mesenteroides* can produce isomaltooligosaccharides (IMO) which selectively stimulate growth of beneficial bacteria such as lactobacilli and bifidobacteria when grown with sucrose and maltose as carbon sources (6). *Lb. casei* could also produce oligosaccharides after milk fermentation when co-cultured with *Lactococcus lactis* ssp. *diacetylactis* and *Lactobacillus acidophilus*. During fermentation, they produced β-galactosidase presenting lactose hydrolysis and transgalactosidal activities (7).

To date, many studies have focused on the development of compounds as BGSs, including prebiotics (8), whereas lactic acid bacteria (LAB), which can directly stimulate the growth of bifidobacteria, have rarely been studied. If LAB can directly stimulate bifidobacteria, they can be used as enhancers for bifidobacterial growth during their cultivation or storage. Therefore, we investigated whether *Leu. mesenteroides* CJNU 0147 and *Lb. casei* CJNU 0588 presenting BGS acitivty via whey fermentation (4,5) can directly stimulate a bifidobacterial strain in co-cultures in this study.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Leu. mesenteroides CJNU 0147 and *Lb. casei* CJNU 0588 strains were cultured in MRS broth (Difco, Sparks, MD, USA) at 37°C without agitation. *Bifidobacterium lactis* BL 750 (Culture Systems, Mishawaka, IN, USA) was cultured in reinforced clostridial medium (RCM; Difco, Detroit, MI, USA) broth at 37°C in an anaerobic jar (Oxoid Co., Cambridge, UK).

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Co-cultures

To investigate whether *Leu. mesenteroides* CJNU 0147 and *Lb. casei* CJNU 0588 can directly stimulate the growth of *B. lactis* BL 750 strain, co-cultures were performed in both RCM and whey broth (10%, w/v). *Leu. mesenteroides* CJNU 0147 and *Lb. casei* CJNU 0588 were cultured (approximately 10^9 cells/mL) in 5 mL of MRS broth for 24 hr at 37°C and each culture was added to RCM broth at a final concentration of 0.1%. The RCM had been previously inoculated with *B. lactis* BL 750 strain (approximately 10^9 cells/mL) with a final concentration of 0.5%. The mixed cultures were incubated at 37°C for 24 hr in an anaerobic jar (Oxoid) and the viable cell count of the *B. lactis* strain was measured at designated times on RCM agar plates under anaerobic condition. To contrast the bifidobacterial strain with added *Leu. mesenteroides* CJNU 0147 or *Lb. casei* CJNU 0588, colonies on the plates were confirmed using two methods: by aerobic culture using MRS broth where only facultatively anaerobic LAB strain can grow and by polymerase chain reaction (PCR) using bifidobacterial specific primers Bif164 (5'-GGGTGGTAATGCCGGATG-3') and Bif662 (5'-CCACCGTTACACCGGGAA-3') (9). Both strains were also added to whey broths (10%, w/v), as final concentrations of 0.1%; each broth had been previously inoculated with the *B. lactis* strain as described above. Viable cells were enumerated as described above.

Survival of *B. lactis* BL 750 strain in MRS broth not in anaerobic jar

To explore the use of fermented whey containing live cells of *Leu. mesenteroides* CJNU 0147 and *Lb. casei* CJNU 0588 as additives to dairy products such as fermented milk, we investigated if the viable bacteria could improve the survivability of *B. lactis* BL 750, which is often added to fermented milk as a probiotic. Fermented whey was produced by using CJNU 0147 and CJNU 0588 and was added to 5 mL of MRS broth previously inoculated with *B. lactis* BL 750 strain. The resulting inoculum was stored at 10°C and viable cell counts of *B. lactis* BL 750 strain were periodically determined (0, 1, 2, 3, 4, 5, 6, 7, 10, 15 days). Briefly, 10-fold diluents were spread on RCM agar plates and anaerobically incubated at 37°C for 24 hr and colonies were counted. To confirm *B. lactis* BL 750 strain, aerobic culture and PCR using bifidobacterial specific primers Bif164-Bif662 were used as described above.

RESULTS AND DISCUSSION

Bifidobacterial growth stimulation activity of viable *Leu. mesenteroides* CJNU 0147 and *Lb. casei* CJNU 0588 was determined in RCM broth, which is an optimal medium for bifidobacteria. Up to three hours of growth, there were no changes in the growth rates among the samples (i.e., *B. lactis* alone, *B. lactis* + CJNU 0147 or *B. lactis* + CJNU 0588 cells). After three hours, however, the growth rates of *B. lactis* BL 750 strain with CJNU 0147 or CJNU 0588 cells were slightly increased compared to that of control (*B. lactis* alone). The difference was the greatest at 12 hours, with the viable cell count of the control at 8.4 log cfu/mL, while the viable cell counts of *B. lactis* BL 750 cultured with CJNU 0147 or CJNU 0588 cells reached 8.9 log cfu/mL (Fig. 1). After that time, the viable cell counts of the control and test samples were almost the same. Since RCM broth is an optimal medium for the growth of bifidobacteria, it is difficult to differentiate the growth rates between the control and test samples. Nevertheless, both CJNU 0147 and CJNU 0588 strains could improve the growth of *B. lactis* BL 750 strain in the medium during the exponential phase, which was a very promising result. So, we next applied the same experimental protocol to whey broth (10%, w/v), which is not an optimal medium for the growth of bifidobacteria. This experiment could readily confirm any BGS activity of CJNU 0147 and CJNU 0588 cells. As expected, unlike RCM broth, *B. lactis* BL 750 strain grew very slowly in the whey broth over 24 hours. Up to three hours of growth, there was no change between the control (*B. lactis* alone) and test samples (*B. lactis* with CJNU 0147 or CJNU 0588 cells). At six hours, viable cell counts of the test samples were higher than that of the control. The count of the control reached 6.36 log cfu/mL, whereas those of *B. lactis* with CJNU 0147 or CJNU 0588 reached 6.80 and 6.67 log cfu/mL, respectively. At 12 hours, the difference between the control and samples was the greatest of the entire culture period. The cell number of the control

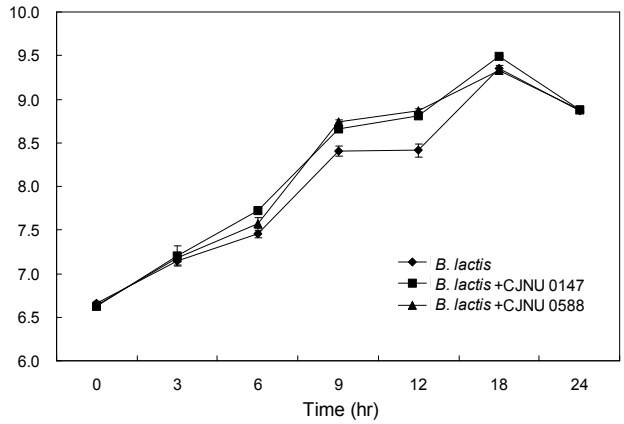

Fig. 1. Viable cell counts of *Bifidobacterium lactis* BL 750 strain co-cultured with *Leuconostoc mesenteroides* CJNU 0147 or *Lactobacillus casei* CJNU 0588 in RCM broth.

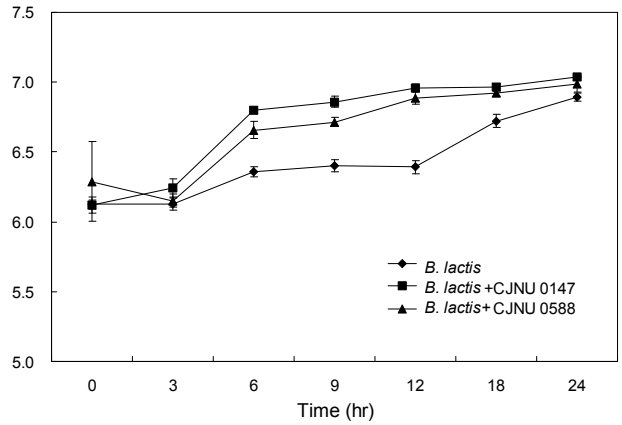

Fig. 2. Viable cell counts of *Bifidobacterium lactis* BL 750 strain co-cultured with *Leuconostoc mesenteroides* CJNU 0147 or *Lactobacillus casei* CJNU 0588 in whey broth.

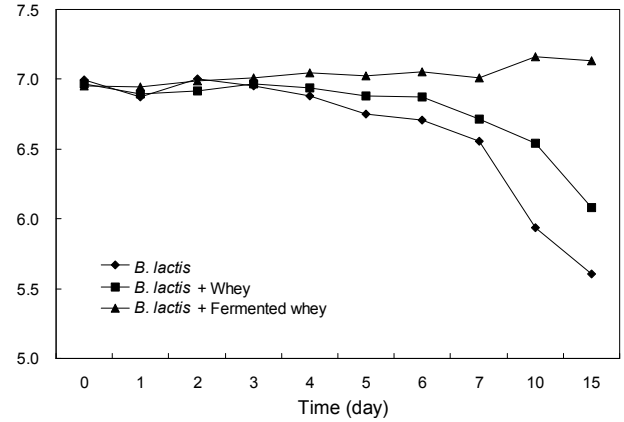

Fig. 3. Viable cell counts of *Bifidobacterium lactis* BL 750 strain stored in MRS broth at 10°C with fermented whey. The fermented whey was produced by *Leuconostoc mesenteroides* CJNU 0147 and *Lactobacillus casei* CJNU 0588 strains. Whey was also added as a control sample.

reached 6.39 log cfu/mL, whereas those of *B. lactis* with CJNU 0147 or CJNU 0588 reached 6.96 and 6.89 log cfu/mL, respectively (Fig. 2). Unlike with RCM broth, even after 24 hours the viable cell counts of the samples were still higher than that of the control. These results prove that viable *Leu. mesenteroides* CJNU 0147 and *Lb. casei* CJNU 0588 cells can directly improve the growth of a bifidobacterial strain. Therefore, these cells could be used as bifidobacterial growth enhancers, though their efficacy should be re-confirmed under various conditions.

In previous reports, cell-free fermented whey prepared with CJNU 0147 or CJNU 0588 was confirmed to have BGS activity (4,5). This may have reflected the production of metabolites from CJNU 0147 or CJNU 0588 during whey fermentation. In this study, we applied viable CJNU 0147 or CJNU 0588 cells to co-cultures with *B. lactis* BL 750. This is different from the experimental condition in prior studies, because CJNU 0147 or CJNU 0588 cells might compete for survivability with the *B. lactis* strain. Vinderola et al. (10), investigated interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. From this study, they categorized four different kinds of behaviors between species of lactic acid starter and probiotic bacteria: stimulation, delay, complete inhibition of growth, and no effect between them. Generally, lactic acid bacteria did not show any effect on the growth of the probiotic bacteria, such as lactobacilli and bifidobacteria, with some exceptions. Promisingly, our strains of CJNU 0147 and CJNU 0588 might be compatible with probiotic bifidobacteria in fermented milk products, which can enlarge application of the strains to dairy industry. Additionally, fermented whey containing viable cells of CJNU 0147 and CJNU 0588 maintained the viability of the *B. lactis* strain stored aerobically at 10°C in MRS broth. Specifically, the viable cell count of the *B. lactis* strain stored without the fermented whey decreased to 5.6 log cfu/mL after 15 days, whereas that of the strain stored with the fermented whey was slightly increased to 7.1 log cfu/mL, as compared with the initial viable cell count of 6.9 log cfu/mL (Fig. 3). Normally, the viable cell counts of bifidobacteria would be decreased in an air-permeable condition since they are strict anaerobes (11); however, fermented whey containing viable CJNU 0147 and CJNU 0588 cells could maintain the viability of *B. lactis* BL 750 strain, indicating it could be used as a bifidobacterial-preserving agent in air-permeable conditions, such as yogurt products.

In conclusion, *Leu. mesenteroides* CJNU 0147 and *Lb. casei* CJNU 0588, as well as fermented whey prepared with both strains, can directly stimulate the growth of bifidobacterial strain *B. lactis* BL 750, which is commercially applied to fermented milk products as a probiotic strain. Also, the fermented whey containing live cells of CJNU 0147 and CJNU 0588 maintained the viability of the bifidobacterial strain during storage at 10°C under aerobic condition. These results are intriguing and suggest both strains could be promising for commercial uses. In near future, we will identify the bifidogenic growth stimulators from CJNU 0147 and CJNU 0588 strains.

ACKNOWLEDGEMENT

This research (grant no. 110111-1) was supported by the Technology Development Program for Food, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

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(Received October 18, 2011; Accepted December 15, 2011)