J. Microbiol. Biotechnol. (2001), **11**(1), 97–105

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Estimating the Viability of *Bifidobacterium longum* in Ca-Alginate Beads Against Simulated Gastroenteric Juices

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Received: September 16, 2000
Accepted: January 2, 2001

Abstract The viability of *Bifidobacterium longum* KCTC 3128, entrapped in calcium alginate beads in simulated gastroenteric juices (gastric and bile salt solution), was tested to evaluate the influences of several parameters (gel concentration, bead size, and initial cell number). The death rate of *B. longum* in beads after being sequentially exposed to simulated gastric juices and bile salt solution decreased proportionally with increasing both the alginate gel concentration and bead size. The number of initial cell loading in beads affected the numbers of survivors after being exposed to these solutions, while the death rate of the viable cells were not affected. From the results obtained, the influence of entrapment parameters on the survival of bifidobacteria was quantitatively and systematically evaluated by using a mathematical method.

Key words: *Bifidobacterium longum*, Ca-alginate, entrapment, gastroenteric juices, mathematical model

Bifidobacteria constitute a major part of the intestinal microflora of humans and various animals [15, 33]. Twenty-eight species have so far been isolated from the various origins, and 10 of them are of human origin [3, 22]. When present in sufficient numbers, these organisms may play an important role in restricting the growth of many potential pathogens and putrefactive bacteria in the gastrointestinal tract. Anticarcinogenic and anticholesterolemic properties of the organisms were also reported [9, 13]. Due to their therapeutic effects, these bacteria have been added to several dairy products such as fermented milk, cheeses, and health foods [16]. To claim probiotic effects of the bifidobacteria included in the dairy products, they must colonize the colon of hosts. Following ingestion, the organisms must survive in transit through the gastric environment and eventually reach the colon in quantities large enough to facilitate colonization. When dairy products containing bifidobacteria were given therapeutically to humans, the viability of bifidobacteria was rapidly lost in the gastric environment within one hour [26]. The germicidal effect of gastric juices was mainly attributable to its low pH level, which might play an important role [7, 8]. Although a number of viable bifidobacteria passed through the stomach, other factors such as digestive enzymes or bile salts might be harmful to the viability of the cells [24]. It was reported that the microencapsulation of bifidobacteria could be used as methods to ensure greater survival under the gastric and intestinal conditions [26]. Immobilized cells provided many advantages over free cells as follows: maintenance of stable and active biocatalysts, high volumetric productivity, improved process control, protection of cells against damage, and reduced susceptibility to contamination [20, 27]. Recently, yogurt product containing the encapsulated lactic acid bacteria has been distributed as Doctor-Capsule (Bingrae Co., Korea) in Korea. Among various techniques for immobilizing living cells, entrapment in Ca-alginate beads has commonly been used [30]. Alginate is nontoxic to cells and it is an accepted food additive [25].

In the present paper, the influences of alginate concentration, bead size, and initial cell numbers on the survival rate of the entrapped *Bifidobacterium longum* against simulated gastric juices and bile salts are represented by using a mathematical calculation.

MATERIALS AND METHODS

Bacteria and Media

Bifidobacterium longum KCTC 3128 (ATCC 15707) in a lyophilized form was purchased from the Korea Collection

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for Type Cultures (Genetic Resource Center, Taejon, Korea). This bacterium was transferred twice in TPY (trypticase-proteose peptone-yeast extract) broth at 37°C. Cultivation was carried out in a 2.5-l fermenter containing 1,000 ml of TPY broth. After cultivation for 20 h, cultures were collected by centrifugation (3,000 ×g, 10 min), washed, and resuspended in 0.85% NaCl saline solution to make approximately 10^9 cells/ml.

Entrapment of Cells

Sodium alginate (medium viscosity) obtained from Sigma Chemical Co. was used in this study. Sodium alginate (5, 7.5, and 10 g) was autoclaved at 121°C for 15 min in powder, and then separately dissolved in 250 ml each of the resuspended cell solution in the aseptic vinyl bag. The solution was thoroughly mixed by using the laboratory blender stomacher 400 (Seward Co., U.K.). Using the aseptic and compressed air which was filtered through autoclaved filters (5, 1, and 0.22 μm) which were sequentially set in the compressor, the cells-alginate mixture was ejected dropwise through a 20G nozzle into 0.1 M of $CaCl_2$ solution in a clean bench. The bead size could be controlled by varying the compressed air pressure. The resulting calcium alginate beads were cured for 1 h in a 0.1 M $CaCl_2$ solution, washed with sterile saline solution, and stored in TPY broth at 4°C before use. Diameters of calcium alginate beads were measured with the eyepiece micrometer in the optical microscope at a magnification of 100×. At least 100 randomly selected beads were measured for each sample.

Viability of the Entrapped Cells

Simulated gastric juices without pepsin (0.08 M HCl containing 0.2% NaCl, pH 1.55) were prepared by the method of Rao [26]. One-hundred samples of beads were separately added to six cap-tubes containing 10 ml of simulated gastric juice and incubated anaerobically at 37°C for 3 h. In order to determine the effect of bile salts on the survival of bifidobacteria, one-hundred samples of beads were separately added to three cap-tubes containing 10 ml of bile salt solution that was dissolved in 0.6% oxgall (Difco, Co.) which had been autoclaved at 121°C for 15 min, then incubated anaerobically at 37°C for 6 h. During incubation, all beads were harvested from one cap-tube sample at various intervals (30 min in simulated gastric juices, 2 h in the bile salt solution), washed with physiological saline, and immediately assayed for the cell enumeration. In order to prepare samples for untrapped *B. longum*, 10 ml of cultures were centrifuged (10 min, 3,000 ×g). The pellets were resuspended in 0.85% saline solution, and collected by centrifugation. Supernatants were discarded, and a 10 ml of simulated gastric juices and a 10 ml of the bile salt solution were added into each cap-tube containing the recovered cells. Then, the cells were lightly agitated, incubated at 37°C for the same period of time as described for the entrapped cells, and assayed for the cell enumeration. Effects of initial cell loading in beads on the survival of *B. longum* were tested. Cultivated cells were centrifuged and then resuspended in 0.85% of NaCl saline solution to make approximately 10^8, 10^9, and 10^{10} cells/ml. Using this cell suspension, three different initial cell-loaded beads containing 3% alginate with 2.62 mm were prepared. One-hundred samples of each type of beads were separately added to six cap-tubes containing 10 ml of simulated gastric juice and incubated anaerobically at 37°C for 3 h. In addition, *B. longum* entrapped in 3% alginate beads with 2.62 mm were sequentially exposed to simulated gastric juices followed by the bile salt solution. One-hundred samples of these beads were separately added to six cap-tubes containing 10 ml of the simulated gastric juice and incubated anaerobically at 37°C for 3 h. During incubation, all beads were harvested from one cap-tube sample at intervals of 1 h, washed with physiological saline, and immediately assayed for the cell enumeration. After 3 h, the solution in the other remaining three cap-tubes was changed from simulated gastric juices to 10 ml of the bile salt solution. During incubation, all beads were harvested from one cap-tube sample at intervals of 2 h, washed with physiological saline, and immediately assayed for the cell enumeration.

Cell Enumeration

The total viable numbers of untrapped *B. longum* expressed as colony forming units (cfu) were determined by using the plate count method with TPY agar. In order to count the viable cell numbers in beads, 100 particles were washed with sterile saline solution and dissolved in 30 ml of sterile 0.1 M sodium citrate solution with the aid of a stomacher 400 for 10 min, and then the viable cells of *B. longum* in one milliliter of the dissolving solution were determined by using the plate count method with TPY agar. Cultivation of bifidobacteria on the TPY agar plate was carried out in the anaerobic system (Forma Scientific Inc., U.S.A.) filled with the mixed gases consisting of N_2 (75%), H_2 (10%), CO_2 (5%) at 37°C for 48 h.

RESULTS

Bead Size and Distribution

B. longum was entrapped in calcium alginate beads containing 2%, 3%, and 4% sodium alginate. Large-, medium-, and small-sized beads (mean diameters; about 1.03, 1.75, and 2.62 mm, respectively) were obtained from each concentration of calcium alginate by controlling the compressed air pressure at the 20G nozzle (Table 1). Diameters of 100 beads from a representative sample of each preparation were measured with an eyepiece micrometer under an optical microscope. Figure 1 shows the size distribution of

Table 1. Preparation of various size beads containing 2%, 3%, and 4% alginate concentration.

Alginate (%)	Nozzle size	Air pressure (kg/cm^2)	Mean bead diameter (mm)
	20G	3.3	1.03
2	20G	2.8	1.73
	20G	2.3	2.59
	20G	3.5	1.03
3	20G	3.0	1.75
	20G	2.5	2.62
	20G	3.8	1.04
4	20G	3.3	1.76
	20G	2.8	2.61

beads which entrapped *B. longum*, and the significance is that the size of beads produced, for the most part, was closely dispersed to each average bead diameter. As shown in Fig. 2, three different sizes of beads obtained from 3% sodium alginate solution were shaped like a spherical ball, and the shape of other beads prepared from 2% and 4% sodium alginate solution were also spherical (data not shown). These results indicate that bead samples have a relative uniformity in their size and shape.

Survival of Bifidobacteria in Simulated Gastric Juices

B. longum entrapped in large-, medium-, and small-sized beads was separately exposed to simulated gastric juices at 37°C for 3 h. Figure 3 shows the results of the viable cell numbers of entrapped *B. longum* after being exposed to simulated gastric juices. When these alginate beads were exposed to simulated gastric juices for 3 h, the cell viability in beads decreased proportionally and logarithmically. The concentration of alginate had a significant effect on cell viability; the higher the concentration of alginate in the three-sized beads, the lower the death rate (slope of line) of *B. longum* entrapped in the beads. In addition, the death rate of cells in the beads decreased proportionally with increasing the size of beads (Fig. 3A). The viable cell numbers of untrapped *B. longum* rapidly decreased from 1.28×10^9 cfu/ml to below 10^3 cfu/ml within 30 min. Therefore, in the presence of simulated gastric juices, the survival of entrapped *B. longum* was higher than that of the untrapped cells. These results also indicate that there is an interrelationship between the death rate of entrapped bifidobacteia, the alginate concentration, and the size of beads. The change of viable cell numbers in beads (death rate) is given by dN_g/dt_g, where N_g is the log viable cell numbers in the beads at a certain alginate concentration level and bead size in the presence of simulated gastric juices, and t_g represents the exposure time of the simulated gastric juices. Assuming that the beads have a constant cell density ρ, the log viable cell numbers of bifidobacteria in the beads can be given by the following equation:

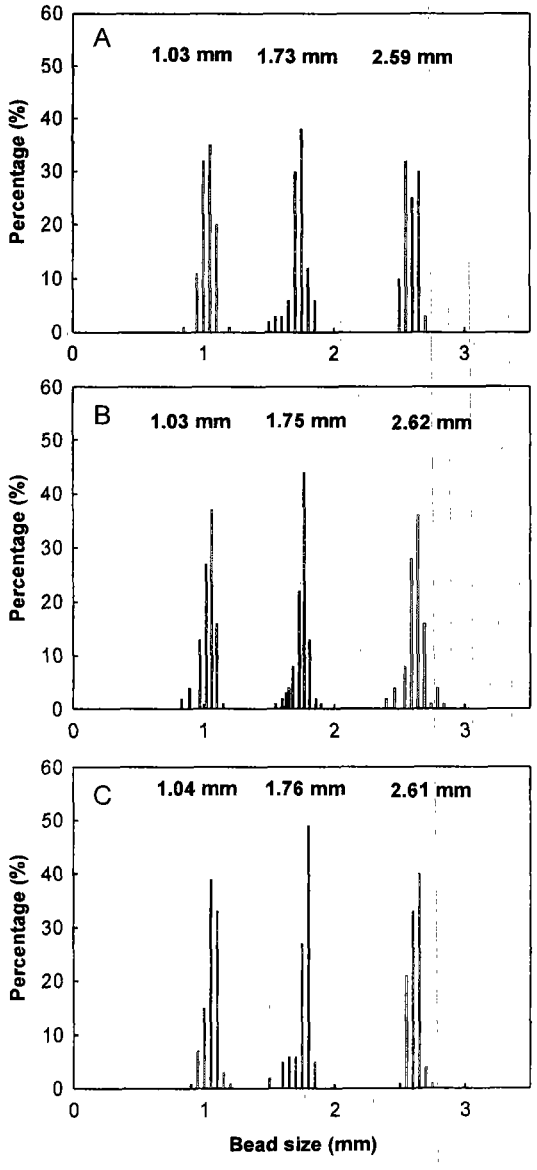

Fig. 1. Size distribution of several alginate beads entrapping *B. longum* KCTC 3128.
(A) 2% alginate beads; (B) 3% alginate beads; (C) 4% alginate beads.

$$N_g=\left(\frac{dN_g}{dt_g}\right)t_g+N_{go} \quad (1)$$

where N_{go} is the log viable cell numbers in the beads before being exposed to simulated gastric juices. In Fig. 3B, the death rate of cells in the beads decreased proportionally with the increasing alginate concentration of beads. Therefore, a change of the cell death rate in the beads after being exposed to simulated gastric juices by the change of alginate concentration can be written as,

$$\frac{\partial}{\partial C}\left(\frac{\partial N_g}{\partial t_g}\right)=\alpha_g \quad (2)$$

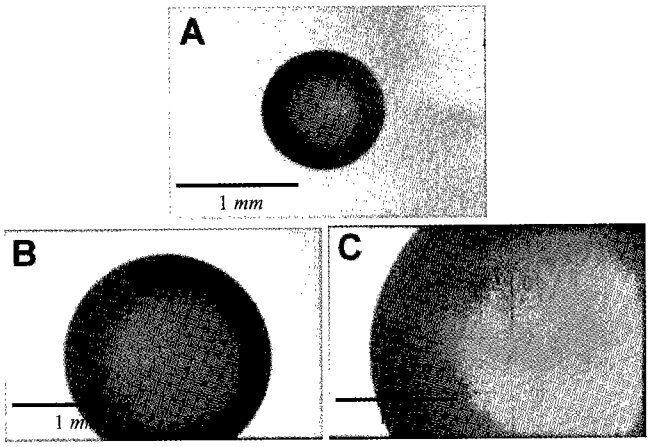

Fig. 2. Photographs of beads entrapping *Bifidobacterium longum*.
A: 1.03-mm diameter bead with 3% alginate; B: 1.75-mm diameter bead with 3% alginate; C: 2.62-mm diameter bead with 3% alginate.

where C is the alginate concentration of beads and α_g is a slope of cell death rate in the beads. In Fig. 3C, the value of α_g increased proportionally with increasing bead size. Therefore, the change of α_g by the change of bead size can be written as

$$\frac{d\alpha_g}{dS}=\beta_g \tag{3}$$

where S is the bead size and β_g is the slope of α_g. Thus, from the equations (1), (2) and (3), the viable cell numbers in the beads at a certain alginate concentration and bead size in the presence of simulated gastric juices can be expressed as follows:

$$N_g=\left\{[(\beta_g S+\alpha_{gso})C]+\left(\frac{dN_g}{dt_g}\right)_{co}\right\}t_g+N_{go} \tag{4}$$

where α_{gso} is the slope of cell death rate when S is zero, and $(dN_g/dt_g)_{co}$ is the change of viable cell numbers in the beads when C is zero. These three values including β_g were calculated from the experimental data. However, $(dN_g/dt_g)_{co}$ also changed with the change of the bead size (Fig. 3D). This change is expressed as

$$\frac{\partial}{\partial S}\left(\frac{dN_g}{dt_g}\right)_{co}=\gamma_g \tag{5}$$

where γ_g is the slope of $(dN_g/dt_g)_{co}$. Equation (5) can be integrated as

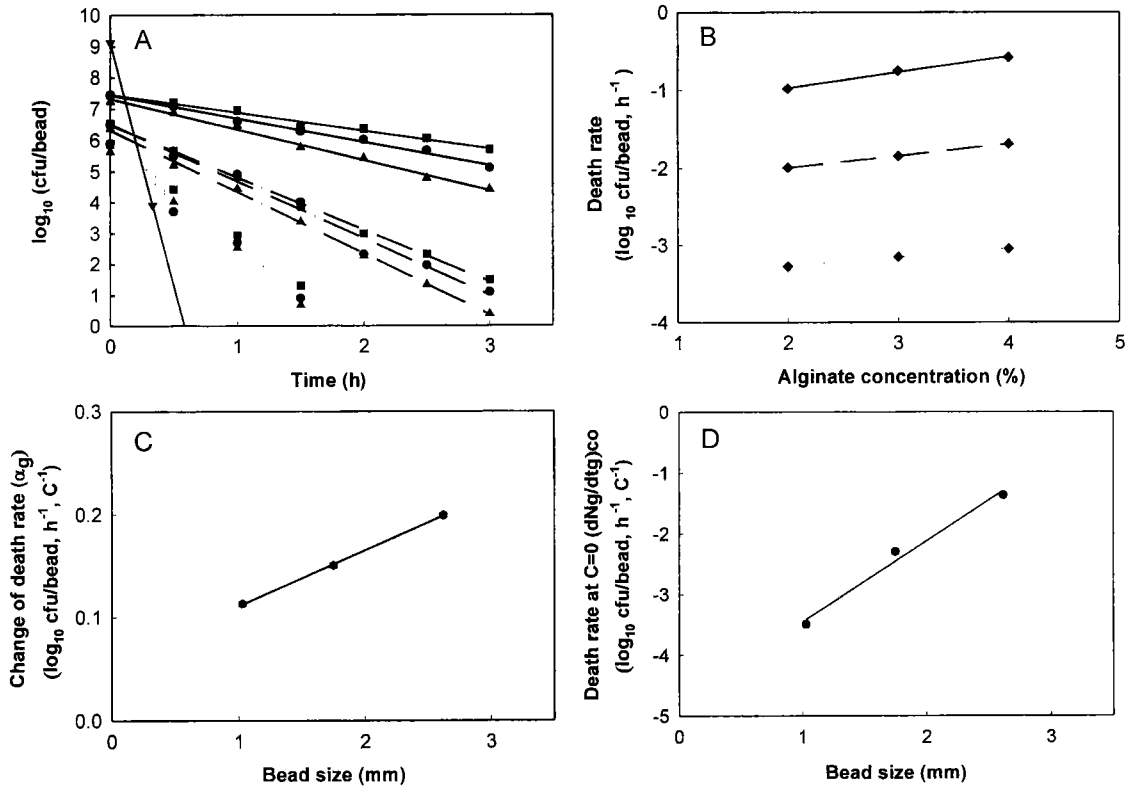

Fig. 3. Survival of entrapped *Bifidobacterium longum* after being exposed to simulated gastric juices at 37°C for 3 h.
(A) The viable cell number of entrapped *B. longum*, ▲: 2% alginate beads; ●: 3% alginate beads; ■: 4% alginate beads; -▼-: untrapped *B. longum*; ···: small-sized beads; ---: medium-sized beads; – : large sized beads. (B) The death rate of *B. longum* in beads, ···: small-sized beads; ---: medium-sized beads; – : large sized beads. (C) The change of death rate of *B. longum* in beads by the change of bead size. (D) The change of death rate when alginate concentration is zero by the change of bead size.

$$\left(\frac{dN_g}{dt_g}\right)_{co}=\left(\frac{dN_g}{dt_g}\right)_{cos\,o}+\gamma_g S \quad (6)$$

where $(dN_g/dt_g)_{coso}$ is $(dN_g/dt_g)_{co}$ when S is zero. Therefore, Eq. (4) can be expressed as follows:

$$N_g=\left\{[(\beta_g S+\alpha_{gso})C]+\left(\frac{dN_g}{dt_g}\right)_{soco}+\gamma_g S\right\}t_g+N_{go} \quad (7)$$

When 3% of the alginate beads with 2.62-mm diameter containing different N_{go} were exposed to simulated gastric juices, N_{go} (the number of initial cell loading in beads) affected N_g (the numbers of survivors) (Fig. 4A). However, dN_g/dt_g, recognized as the death rate of the viable cells, was not affected (Fig. 4B). In addition, the death rates estimated from the theoretical data were not in disagreement with the death rates estimated from the experimental data. Thus, it was demonstrated that each bead containing bifidobacteria had a constant cell density (ρ), and viable cells proportionally decreased from the surface of the bead into the core.

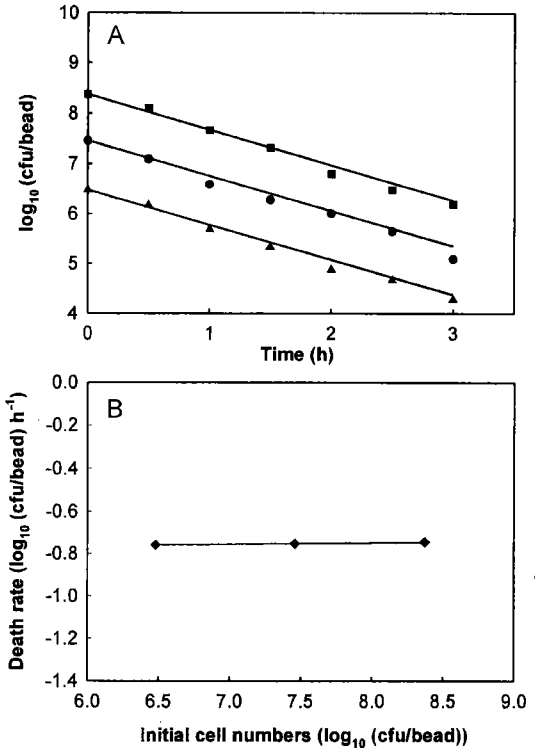

Fig. 4. Theoretical and experimental survival of *B. longum* KCTC 3128 entrapped in 3% alginate beads containing three different initial cell numbers after being exposed to simulated gastric juices at 37°C for 3 h.
(A) Entrapped *B. longum* in 3% calcium alginate beads; (B) the change of the death rate of entrapped bifidobacteria by the change of initial cell numbers in beads. The lines (---) are calculated using Eq. (10). The points (●, ■, ▲, and ◆) are experimental data. -▲-, 3.02×10^6 cfu/bead of initial cell numbers; -●-, 2.88×10^7 cfu/bead of initial cell numbers; -■-, 2.35×10^8 cfu/bead of initial cell numbers; -◆-, death rate of *B. longum*.

Therefore, it can be stated that this equation model (7) had some credibility.

Survival of Bifidobacteria in Bile Salt Solution

Survival of *B. longum* entrapped in large-, medium-, and small-sized beads in the presence of bile salt solution was separately examined at 37°C for 6 h. As expected, the loss of cell viability in all of the alginate beads occurred proportionally and logarithmically, as shown in Fig. 5. The alginate concentration had a mild effect on cell viability (Fig. 5A). Nevertheless, the cell death rate in beads decreased proportionally with increasing the alginate gel concentration and the bead size (Fig. 5B). Alive untrapped *B. longum* decreased from 1.10×10^9 cfu/ml to 3.63×10^5 cfu/ml after being exposed to bile salt solution. Therefore, the survival of entrapped bifidobacteria in the bile salt solution can also be expressed in the manner of the above Eq. (7). It is defined as follows:

$$N_g=\left\{[(\beta_b S+\alpha_{bso})C]+\left(\frac{dN_b}{dt_b}\right)_{cos\,o}+\gamma_b S\right\}t_b+N_{bo} \quad (8)$$

where N_b is the log viable cell numbers in beads at a certain alginate concentration and bead size in the presence of bile salt solution, and t_b is the exposure time of the bile salt solution. Also, α_b is defined as the slope of the cell death rate in beads after being exposed to the bile salt solution by the change of alginate concentration, and β_b is the slope of α_b by the change of bead size (Fig. 5C). As shown in Fig. 5D, γ_b is the slope of $(dN_b/dt_b)_{co}$. β_b, α_{bso}, γ_b, C_o, and N_{bo} were calculated from the experimental data.

However, in the case where some entrapped *B. longum* is sequentially exposed to simulated gastric juices and bile salt solution, N_{bo}, which the log viable cell numbers before being exposed to the bile salt solution in Eq. (8), is the same as N_g, the log viable cell numbers in the beads after being exposed to simulated gastric juices in Eq. (4). Although the bead size is externally unchangeable in the presence of simulated gastric juices, the actual bead volume occupied with the viable cells becomes continuously reduced. Thus, the S bead size of Eq. (5) can not be matched with the intrinsic bead size. Therefore, the following model is proposed to describe the bead size corresponding to the actual volume that was occupied with the viable cells. Since the cells died from the surface of the beads, the actual volume occupied with survivors decreases. What this means is that the actual bead size occupied with survivors becomes smaller. The reduced rate of bead size is given by $-dN_g/dt_g$, where N_g is the log viable cell numbers of beads before being exposed to simulated gastric juices, t_g is the reaction time, and ρ is the cell density of beads. So we have now have

$$-\frac{dN_g}{dt_g}=-\rho\frac{dV}{dt_g} \quad (9)$$

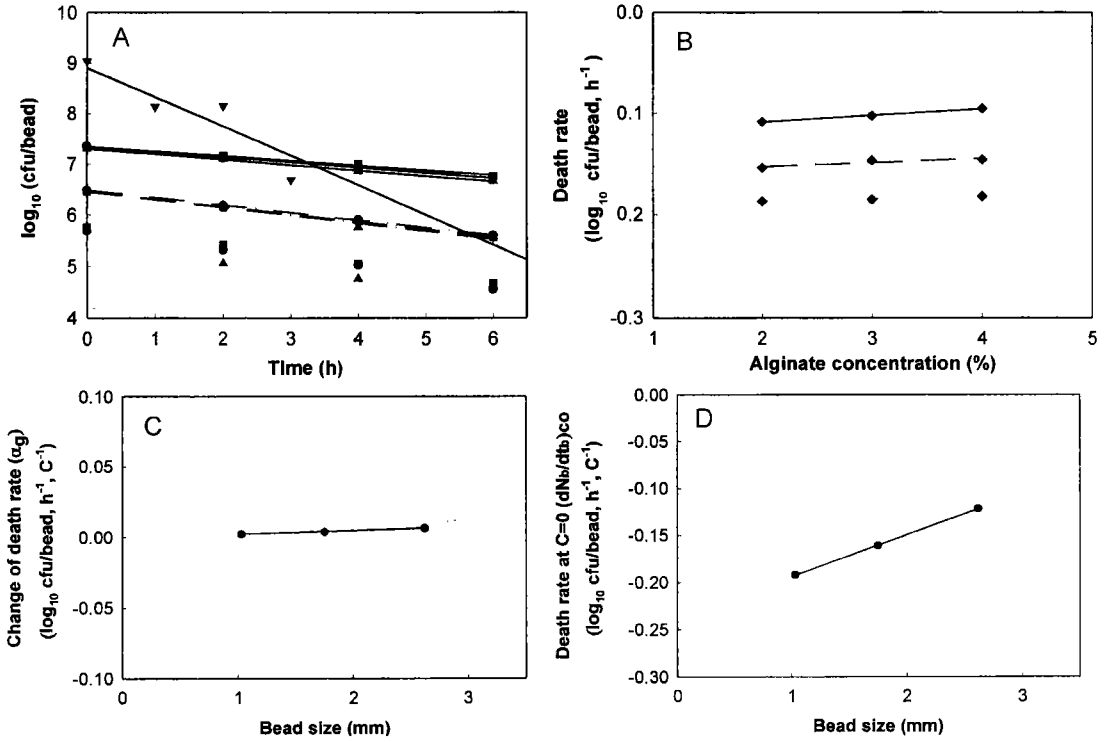

Fig. 5. Survival of entrapped bifidobacteria after being exposed to bile salt solution at 37°C for 6 h.
(A) The viable cell number of *B. longum*, ▲: 2% alginate beads; ●: 3% alginate beads; ■: 4% alginate beads; ···: small-sized beads; ---: medium-sized beads; – : large sized beads; -▼-: untrapped *B. longum*. (B) The death rate of *B. longum* in beads; ···: small-sized beads; ---: medium-sized beads; – : large sized beads. (C) The change of death rate of *B. longum* in beads by the change of bead size. (D) The change of death rate, when alginate concentration is zero, by the change of bead size.

where V is the bead volume occupied with the viable cells. Assuming that the beads have the complete spherical shape, it can be represented by

$$V=\frac{4}{3}\pi R^3 \tag{10}$$

where R is the radius of the bead. Substitution of Eq. (7) into Eq. (6) provides the change of viable cell numbers in the beads. That is,

$$-\frac{dN_g}{dt_g}=-\rho 4\pi R^2\frac{dR}{dt_g} \tag{11}$$

Thus, Eq. (8) can be integrated to give the bead radius as a function of time. It can be rearranged by

$$R=\sqrt[3]{\frac{3}{4\rho\pi}(N_g-N_{go})+R_o^3} \tag{12}$$

where R_o is the initial radius of bead before being exposed to simulated gastric juices. The viable cell numbers in calcium alginate beads after being sequentially exposed to simulated gastric juices and the bile salt solution can be expressed by using Eqs (7), (8) and (12). That is

$$N_g=\left\{[(\beta_g S+\alpha_{gso})C]+\left(\frac{dN_g}{dt_g}\right)_{\cos o}+\gamma_g S\right\}t_g$$

$$+\left\{[(\beta_b S'+\alpha_{bs'o})C]+\left(\frac{dN_b}{dt_b}\right)_{\cos' o}+\gamma_b S\right\}t_b+N_{go} \tag{13}$$

where S' is the actual bead size occupied with the viable cell numbers after simulated gastric juices, that is 2R.

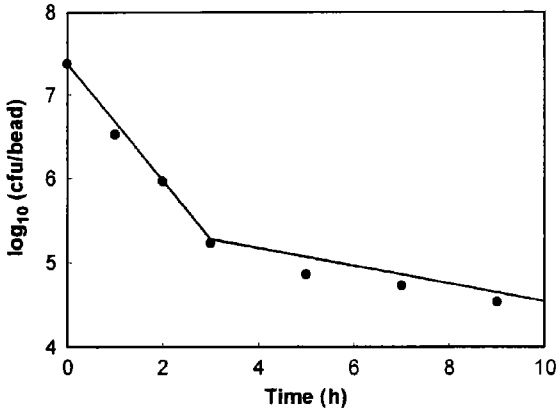

Fig. 6. Theoretical and experimental survival of *Bifidobacterium longum* entrapped in 3% alginate beads containing three different initial cell numbers after being sequentially exposed to simulated gastric juices for 3 h followed by bile salt solution for 6 h.
The line (–) is calculated using Eq. (10). The point (●) is the viable cell numbers of *B. longum* and experimental data.

Figure 5 shows that the number of survivors after being sequentially exposed to simulated gastric juices and a bile salt solution, estimated from Eq. (13) (N_{gb}), is not in disagreement with the measured data. Therefore, this equation has some credibility; however, the experimental data were slightly lower than the calculated data and there was some scatter in the measured data.

DISCUSSION

B. longum ACTC 3128 was entrapped in calcium alginate beads, and the influences of entrapment parameters (alginate concentration, bead size, and initial cell number) on the survival of bifidobacteria were quantitatively and systematically determined. Although there have been some studies on the survival of bifidobacteria and lactic acid bacteria in the presence of gastric and/or intestinal juices [2, 7, 8, 10, 23, 24], this is the first report, to the best of our knowledge, to give mathematical quantification of the survivors of entrapped bifidobacteria after being sequentially exposed to simulated gastric juice and bile salt solution. These mathematical quantifications could be made possible by introducing a new entrapping procedure, where alginate gel containing bifidobacteria was prepared by adding the sterilized sodium alginate powder to the cell suspended solution. This new entrapping procedure provides the uniform cell distribution to the calcium alginate beads. On the other hand, the conventional procedures [5, 21, 25], which are prepared by adding the cell suspension into the sterile sodium alginate solution, provide non-uniform cell distribution in the gel beads. This phenomenon was due to mixing problems; when the inoculum was added to the polymer solution during the bead preparation, this polymer solution of very high viscosity resulted in a non-uniform cell distribution in the gel beads [31]. Sodium alginate concentrations from 2% to 4% were tested in this study. As expected, the higher the concentration of alginate in the beads, the lower was the death rate of cells in the beads. (Figs. 3 and 5). It was reported that the slower diffusion rate of glucose and ethanol in more concentrated alginate gels was due to a decrease in the number and length of the pores rather than a decrease in the pore diameter [12]. Gel concentration below 2% had difficulty in forming a spherical shape of the bead. This effect was in agreement with other investigations [17, 28, 32]. It was shown that low-viscosity droplets have less ability to retain their spherical shape against the drag forces upon collision with the solution. On the other hand, a high concentration of sodium alginate (above 5%) cannot form small-sized droplets because of its physiological characteristics as a dough. Accordingly, cell entrapment will be limited to the possible range of gel concentration to form spherical beads. Alginate is extracted from various families of the Phaeophyceae class (brown seaweeds) [6]. While all alginates are composed of mannuronic acid (M) and guluronic acid (G) subunits, the specific physical properties of the polysaccharides depend on its monomer distribution. More specifically, polyguluronic acid chains crosslink more strongly in the presence of calcium salts than polymannuronic or mixed chains [28]. Commercial alginate solutions have a very wide range of viscosities in a balance between M and G, so that they have a different range of alginate concentrations for forming the spherical bead. Although cell entrapment is carried out by using various commercial alginates, with an exception to the one that was used in this experiment, it is expected that each reaction model to simulated gastric juices and bile salts can be expressed in the same manner, as Eq. (13) showed in this paper. In any case, Eq. (13) can be applied within the limits of alginate concentration in which it is possible to form a spherical shape of the bead.

As shown in Figs. 3 and 5, survival of cells in beads is higher with a larger size of beads. Sheu *et al.* [29] indicated that larger bead diameters provided more protection for *Lactobacillus bulgaricus* in frozen desserts. However, extremely large-sized beads might cause coarseness of texture for living microbial feed supplements, whereas small-sized beads did not provide sufficient protection for the bacteria. Thus, bifidobacteria should be entrapped in a limited range of bead sizes.

When 3% alginate bead with 2.62-mm diameter containing different initial cell loading numbers (N_{go}) of *B. longum* was exposed to simulated gastric juices, N_{go} affected the numbers of survivors (N_g) (Fig. 4A). However, the death rate of the viable cells (dN_g/dt_g) were not affected (Fig. 4B).

On the other hand, Fig. 4 showed that initial cell loading numbers (N_{go}) affected the numbers of survivors (N_g). However, the death rates of the viable cells (dN_g/dt_g) were not affected. Therefore, in order to survive and reach the colon in quantities large enough to facilitate colonization, a great number of initial cells should be entrapped in the beads. Hannoun and Stephanopoulos [12] reported that a higher cell loading weakened the gel. Moreover, there is another problem that must be considered in applying the a higher cell loading. It is the difficulty of reaching a high concentration level of a cultivation of bifidobacteria.

Berrada *et al.* [2] demonstrated that not all commercial bifidobacteria fermented milks could provide human intestine with enough living bifidobacteria to bring about total health benefit. In order to overcome this problem, many investigators have chosen more resistant *Bifidobacterium* strains to gastric acid and bile salts [2, 11, 14, 18, 23 24]. In another effective way, Rao *et al.* [26] developed a preliminary procedure for the microencapsulation of *Bifidobacterium pseudolongum* with cellulose acetate phthalate (CAP) using phase separation-coacervation. Their results showed that microencapsulated bacteria were more resistant than unencapsulated bacteria against the sequential stress

with simulated gastric and intestinal juices. Among the techniques used for immobilizing living cells, gel entrapment using natural biopolymers are favored by the majority of workers for various reasons [1, 19], as follows; non-toxicity of the matrix (crucial in view of food-related applications), simplicity of immobilization technique, and high viability and productivity of the immobilized cells. Until now, most immobilization techniques for bifidobacteria or probiotic bacteria have been developed to test a question of whether the immobilized cells would survive better than nonimmobilized cells [4, 5, 26, 29, 30]. Moreover, the results could not give clear information for devising mathematical quantification models for the survival of immobilized cells against stress that was due to damage. This unclear information must have been caused by nonuniform cell distribution and nonidentical size of capsule and bead.

From the present investigation, it was found that the survival of entrapped bifidobacteria was strongly dependent upon various parameters, such as alginate concentration, bead size, and initial cell numbers. The mathematical model described in this article should be useful for evaluating the influence of various parameters on the survival of entrapped bifidobacteria by the sequential stresses in the gastrointestinal tract and for establishing the optimal conditions for entrapment of bifidobacteria.

Acknowledgments

This work was supported by the special program for Biotechnology of Inha University.

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