Food Sci. Biotechnol. Vol. 15, No. 6, pp. 902 ~ 907 (2006)

Food Science and Biotechnology

Effect of Encapsulated Bacteriocin on Acid Production and Growth of Starter Cultures in Yoghurt

Sejong Oh, Ho Jin Heo[1], Dong June Park[2], Sae Hun Kim[3], Sung Je Lee[4], and Jee-Young Imm[5]*

Department of Animal Science, Institute of Agriculture Science and Technology, Chonnam National University, Gwangju 500-757, Korea

[1]Jeonnam Innovation Agency, Muan, Jeonnam 534-700, Korea

[2]Korea Food Research Institute, Seongnam, Gyeonggi 463-746, Korea

[3]Divisions of Food Science, Korea University, Seoul 136-701, Korea

[4]Institute of Food, Nutrition, and Human Health, Massey University, Auckland, New Zealand

[5]Department of Foods and Nutrition, Kookmin University, Seoul 136-702, Korea

Abstract Freeze dried crude bacteriocin was encapsulated within an acid-soluble coating material, Eudragit EPO, using a surface modification technique through a hybridization system. The pH and titratable acidity of control yoghurt were 3.92 and 1.56%, respectively, after 24 hr of fermentation at 42°C, whereas yoghurt containing 500 AU/mL encapsulated bacteriocin exhibited a higher pH (4.37) and lower titratable acidity (1.2%). Yoghurt containing encapsulated bacteriocin had significantly lower titratable acidity when the duration of fermentation (to pH 4.5) and subsequent refrigerated storage (4°C) was longer than 20 days. There were no significant differences in the viability of lactic acid bacteria after 15 hr of fermentation. This suggests that microencapsulated bacteriocin has the potential to control the excessive growth of yoghurt starters caused by temperature abuse or post-acidification.

Keywords: microencapsulation, bacteriocin, post-acidification, yoghurt

Introduction

Yoghurt is continuously gaining popularity worldwide and its consumption has steadily increased over the past decade. This is partly attributable to the consumer's increased awareness of the health-promoting effects of yoghurt, including decreased lactose intolerance and its efficiency as a probiotic carrier (1). Yoghurt is a custard-like product prepared from milk fermented by lactic acid bacteria (LAB), especially *Lactobacillus* (*L.*) *delbrueckii* ssp. *bulgaricus and Streptococcus* (*S.*) *thermophilus*. During fermentation, LAB utilizes lactose as a substrate and converts it to lactic acid. The lactic acid produced raises the acidity and decreases the pH of milk to between 4.2 and 4.5. The increased acidity in turn induces coagulation of milk proteins, resulting in curd-like textured yoghurt with an astringent, slightly tart taste and flavor.

The acidity of yoghurt is closely related to its quality, thus controlling and maintaining an optimal level of acidity during yoghurt fermentation is one of the most important features of yoghurt manufacturing processes. Over-acidification negatively influences the organoleptic quality of yoghurt. If yoghurt fermentation is not controlled properly or stopped during manufacturing, lactic acid production can continue even under subsequent refrigerated storage, and consequently the pH of yoghurt can drop to as low as 3.6 (2, 3). In addition, the survival and growth of certain LAB, especially most strains of bifidobacteria, are significantly reduced by high acid production (4). This over-acidification or post-acidification is mainly due to uncontrolled acid production by strains of *L. bulgaricus* at low pH (5). To avoid excessive acid production, two main approaches have been applied in the dairy industries that involve the pasteurization of fermented products or changing the starter composition (6, 7). As an alternative means, Weinbrenner *et al.* (8) were the first to suggest the potential of bacteriocin as an acid production suppressor in yoghurt making.

Bacteriocins are antimicrobial proteins or peptides produced by strains of certain bacteria that typically inhibit the growth of similar bacterial strains (9). Characterization of bacteriocins from various fermented food products has been continuously carried out (10, 11) and our preliminary study indicated that the bacteriocin produced by *L. plantarum* KU 107 had an inhibitory effect on lactic acid bacteria such as *L. bulgaricus*, *L. fermentum*, and *L. casei* (12). The inhibitory spectrum of this bacteriocin led us to evaluate its potential for use in suppressing excessive acid production during yoghurt fermentation and subsequent refrigerated storage. However, a direct application of bacteriocins in the manufacture of yoghurt might suppress the growth of the starter culture in the early stages of fermentation leading to insufficient growth of live lactic acid bacteria and/or the delay of fermentation time. To overcome these disadvantages, the bacteriocin was encapsulated within an acid-soluble material.

This study was conducted to examine the effect of encapsulated bacteriocin on the growth and acid production of lactic acid bacteria during fermentation and subsequent refrigerated storage. The potential of microencapsulated bacteriocin as a means to control the excessive acidification of yoghurt was thus evaluated.

*Corresponding author: Tel: 82-2-910-4772; Fax: 82-2-911-4771
E-mail: jyimm@kookmin.ac.kr
Received August 1, 2006; accepted October 9, 2006

Materials and Methods

Bacteria Four bacteriocin producing strains, *L. plantarum* KU 107 (12), *Lactococcus (Lc.)* CU216 (13), *L. acidophilus* 30SC (14), and *L. acidophilus* ATCC 4356 (15), were grown for 18 hr at 37°C in MRS broth (Difco Laboratories, Detroit, MI, USA). Five lactobacilli (*L. casei* YIT 9018, *L. bulgaricus* Mar, *L. bulgaricus* ATCC 7994, *L. bulgaricus* LB18, and *L. lactis* ATCC 4797) and 4 streptococci (*S. thermophilus* ATCC 19258, *S. thermophilus* ATCC 51836, *S. thermophilus* M1, and *S. thermophilus* Y1) used as commercial yoghurt starter cultures were grown for useas indicator strains to test the antimicrobial and inhibitory properties of the various bacteriocins. Lactobacilli and streptococci starter cultures were grown in MRS broth for 18 hr at 37°C and in M17 broth (Difco) for 18 hr at 43°C, respectively. All strains were subcultured three times prior to use and all stock cultures were stored at -80°C in 10% skim milk containing 3% lactose.

Bacteriocin activity Each of the 4 bacteriocins produced was tested for inhibitory activity toward the yoghurt starter cultures using the deferred inhibition assay. The spot-on-lawn method was used to determine the antimicrobial activity of crude bacteriocin (16). The bacteriocin activity was quantified by spotting 20 mL aliquots of the samples onto the surface of MRS agar plates inoculated with 1% of the indicator strain (*L. bulgaricus* ATCC 7994). The plates were incubated at 37°C for 24 hr, and the bacteriocin activity (arbitrary units, AU) was determined by the highest 2-fold dilution showing a clear inhibition zone on the MRS agar.

Preparation of crude bacteriocin Each of the four bacteriocin producers was incubated at 37°C for 24 hr in 10% skim milk containing 1% glucose. After incubation, the inoculated medium was centrifuged at 6,000×g for 30 min at 4°C. The supernatant was then treated with ammonium sulfate (Sigma Chem. Co., St. Louis, MO, USA) at a final concentration of 30%(w/v) and stirred gently overnight at 4°C. The pellet containing crude bacteriocin was collected by centrifugation (8,000×g, 30 min, 4°C) of the supernatant and lyophilized using a freeze drier (Labconco Corp., Kansas city, MO, USA).

Encapsulation of bacteriocin Based on its inhibitory activity and specificity toward starter cultures, the crude bacteriocin derived from *L. plantarum* KU 107 was chosen for encapsulation and addition to yoghurts. Freeze dried bacteriocin powder was ground using an A-10 mill (IKA-Labortechnik, Staufen, Germany). The surfaces of the ground bacteriocin particles were coated and reformed with an acid-soluble coating polymer, Eudragit EPO (Degussa Rohm Pharma Polymers, Piscataway, NJ, USA), in a hybridization system (Model NSH-0; Nara Machinery Co., Ltd., Tokyo, Japan). The formulation ratio of 9:1 (w/w, bacteriocin: Eudragit EPO), running time of 3 min, and rotor speed of 12,500 rpm were determined in a preliminary experiment to optimize the encapsulation process. During the process, the temperature of the hybridization chamber was maintained below 30°C by circulating cooled water through a jacket. In the surface reforming process, fine coating materials adhered to the surfaces of the ground bacteriocin particles in a dry state by friction and collision (17).

Yoghurt preparation *L. bulgaricus* and *S. thermophilus* (Culture systems, Mishawaka, IN, USA) were used for yoghurt preparation. Commercial, pasteurized non-fat bovine milk was fortified with skim milk powder to have 12% solid (w/w). The premix was pasteurized at 95°C for 20 min and cooled to 42°C. The pasteurized premix was inoculated with 0.2% starter culture (w/v). The total level of inoculum was approximately 2×10^6 CFU/mL for each starter culture. The encapsulated bacteriocin particles were then added to the inoculated premixes to a concentration of 100 or 500 AU/mL. The inoculated premix containing encapsulated bacteriocin was incubated at 42°C for 24 hr for yoghurt fermentation. For refrigerated storage trials, portions of the same yoghurt samples were incubated at 42°C until the pH reached 4.5, after which the yoghurt samples were placed in a cold room maintained at 4°C to stop fermentation and stored for 30 days. Control yoghurt was also prepared and treated under the same conditions without the addition of encapsulated bacteriocin.

Release characteristics of encapsulated bacteriocin One g of encapsulated bacteriocin was suspended in 100 mL of 50 mM sodium citrate buffer at pH levels ranging from 5 to 8. The resultant suspension was stirred at 100 rpm for 1 hr at 20°C and then centrifuged at 2,000×g for 10 min at 4 °C. Bacteriocin activity was quantified by spotting 20 μL aliquots of the supernatant onto the surface of MRS agar plates inoculated with 1% of the indicator strain (*L. bulgaricus* ATCC 7994). The plates were incubated at 37 °C for 24 hr, and the bacteriocin activity (AU) was determined by the highest 2-fold dilution showing a clear inhibition zone on the MRS agar. The amount of bacteriocin released was expressed as a percentage of the total bacteriocin activity in the sample. Changes in bacteriocin activity during the refrigerated storage (4°C) of encapsulated bacteriocin particles were also determined.

Viable cells, pH, and titratable acidity of yoghurt One g of yoghurt sample was dispersed into 9 mL of 0.1%(w/v) sterile peptone water (Difco). Subsequent serial dilutions were prepared and viable cells were enumerated using the pour plate technique. Total counts of lactic acid bacteria were enumerated on modified MRS agar (pH 7.0) containing 2% β-glycerophosphate (Sigma) by incubating the plates aerobically at 37°C for 48 hr. The pH was measured using a pH meter (Orion pH meter, Cambridge, MA, USA), and the titratable acidity was determined according to the method described by AOAC (18). All samples were analyzed in triplicate.

Microstructure of encapsulated bacteriocin The microstructures of encapsulated bacteriocin powder and freeze dried powder were observed using a scanning electron microscope (Hitachi Ltd., Tokyo, Japan) as described by Park *et al.* (19). The powder samples were coated for 60 sec with gold-palladium in an E-1010 ion sputter coater (Hitachi Ltd.), and the topography of the powders was observed at 15 KV.

Statistical analysis The experimental data were subjected to analysis of variance (ANOVA) using the SAS program (20). When ANOVA revealed a significant effect at $p<0.05$, the data were further analyzed using Duncan's multiple comparison test.

Results and Discussion

Antimicrobial spectrum The bacteriocins produced by four strains of bacteriocin producers, *L. plantarum* KU107, *Lc.* CU216, *L. acidophilus* 30SC, and *L. acidophilus* ATCC 4356, were tested qualitatively for inhibition of the growth of commercial yoghurt starter cultures of *L. bulgaricus* and *S. thermophilus* (Table 1). The bacteriocin produced by *Lc.* CU216 was active against all tested indicators whereas the bacteriocin by *L. acidophilus* ATCC 4356 had inhibitory activity against *L. lactis* only. Bacteriocin produced by *L. acidophilus* 30SC inhibited the growth of both streptococcus and lactobacillus strains, however its activity was more effective towards streptococcus strains. The bacteriocin from *L. plantarum* KU107 showed activity against all tested lactobacillus strains except for *L. casei*, and did not show any inhibitory activity toward streptococcus strains. The crude bacteriocin produced from *L. plantarum* KU107 was therefore selected as an acid production suppressor since *L. bulgaricus* is known to continuously produce acids in yoghurt even during refrigerated storage (5).

The application of crude bacteriocins in food products has some merits from the industrial point of view since it can be considered as a lactic culture rather than an antibiotic. On the other hand, purified bacteriocins such as nisin and pediocin are categorized as antibiotics and their applications in food products are therefore greatly limited in many countries including Japan and Korea.

Microstructure and release characteristics of encapsulated bacteriocin For the successful application of bacteriocin to yoghurt, the crude bacteriocin was encapsulated within an acid soluble polymer, Eudragit EPO, since the direct addition of bacteriocin might cause a dramatic decline of viable cells in yoghurt. The size and morphology of the freeze dried bacteriocin powder produced by *L. plantarum* KU 107 and the corresponding encapsulated bacteriocin particles were examined using SEM (Fig. 1). The freeze-dried powder was coarse and irregular in shape and existed in an agglomerated cluster ranging from small to large aggregates. The encapsulation process resulted in the formation of relatively spherical particles ranging in size from 20 to 100 μm with a smooth surface containing some cavities and ridges as shown in Fig 1b. The smooth surface formed was attributable to the adhesion of coating material, Eudragit EPO, onto the freeze-dried powder surface, suggesting that the encapsulation process was carried out efficiently by the hybridization system.

When encapsulated bacteriocin particles were suspended in sodium citrate buffer at pH levels ranging from 5 to 8, bacteriocin activity was not observed in the supernatant above pH 6 while about 25% of total activity was detected at pH 6 (Fig. 2). Bacteriocin activity was fully manifested at pH 5. These release characteristics were unaffected by the storage of encapsulated bacteriocin particles at 4°C for up to 2 weeks. These results indicate that the release characteristics of bacteriocin from the encapsulated particles are pH dependent. The surface layer of particles coated with Eudragit EPO was insoluble at pH levels

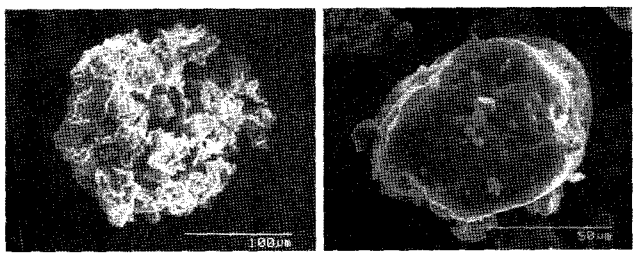

Fig. 1. Scanning electron micrographs of freeze-dried bacteriocin powder (a) and encapsulated bacteriocin particles (b).

Table 1. Inhibitory activity of four bacteriocins toward commercial starter cultures

Indicator strains	Bacteriocin producer[1)]			
	A	B	C	D
L. casei YIT 9018	-[2)]	+	-	-
L. bulgaricus Mar	+	+	+	-
L. bulgaricus ATCC 7994	+	+	-	-
L. bulgaricus LB18	+	+	-	-
L. lactis ATCC 4797	+	+	+	+
S. thermophilus ATCC 19258	-	+	+	-
S. thermophilus ATCC 51836	-	+	+	-
S. thermophilus M1	-	+	+	-
S. thermophilus Y1	-	+	-	-

[1)]A: *L. plantarum* KU107, B: *Lc.* CU216, C: *L. acdidophilus* 30SC. D: *L. acidophilus* ATCC 4356,
[2)]+: inhibition; - : no inhibiton.

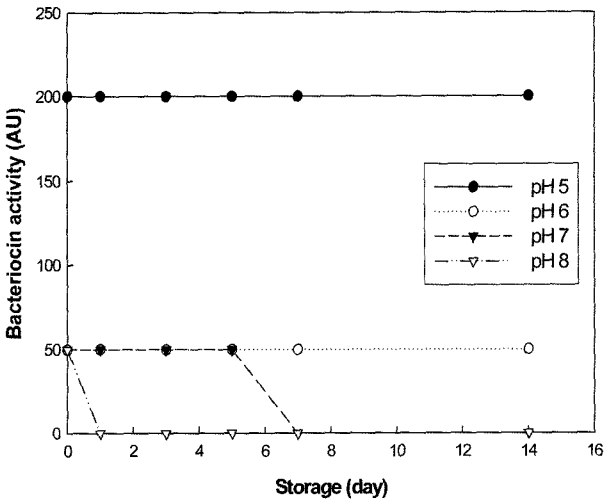

Fig. 2. The pH dependent release pattern of encapsulated bacteriocin during storage at 4°C. AU: arbitrary units.

higher than 6 and provided a water-impermeable barrier to the encapsulated bacteriocins. The low bacteriocin activites (50 AU) detected at pH 6, 7, and 8 are believed to be due to incomplete surface coating and swelling of the surface layer of Eudragit EPO rather than its dissolution, since Eudragit EPO polymer is soluble at pH 5 and below. The pattern of pH-dependent core release observed in this study was consistent with the report of Ohta and Buckton (21) in which Eudragit EPO containing dimethylamino-ethyl groups was dissolved below pH 5.

Changes in pH and titratable acidity of yoghurt during fermentation Changes in the pH of yoghurts during fermentation at 42°C are presented in Fig. 3. Yoghurt starter without bacteriocin produced more lactic acid than starter with encapsulated bacteriocin. After 5 hr of fermentation, the pH of control yoghurt dropped to 4.39 in contrast to 4.78 and 5.75 in the presence of 100 and 500 AU/mL encapsulated bacteriocin, respectively. This indicates that the pH of control yoghurt dropped dramatically in the first 5 hr of fermentation followed by slight decreases afterward. A similar pattern was also observed for yoghurt containing 100 AU/mL encapsulated bacteriocin, although the rate of pH decline was slower. Interestingly, after 24 hr of fermentation, the final pH of this yoghurt sample appeared to be the same as that of control yoghurt. The yoghurt with 500 AU/mL of bacteriocin however had a relatively consistent, slow, steady pH decline over the entire fermentation time, resulting in a higher pH after 24 hr of fermentation relative to the other yoghurt samples.

It should be noted that the time taken to reach the pH 4.5 was 4, 8, and 24 hr for the control and the two yoghurts with 100 and 500 AU/mL of bacteriocin, respectively. This indicates that, although the addition of bacteriocin suppressed acid production in yoghurt, it led to a prolonged fermentation time depending on the level of added bacteriocin. However, the encapsulated bacteriocin might be used along with probiotic strains since *Bifidobacterium* species grow slowly and die quickly at low pH compared with *L. bulgaricus* and *S. thermophilus*. The number of viable cells of *Bifidobacterium* species can be increased with prolonged incubation time if other growth conditions are met. Based on the report of Østlie *et al.* (22), the most desirable sourness and firm coagulum would be expected when the pH of yoghurt was between 4.2 and 4.3. In this regard, the encapsulated bacteriocin could be used to prevent loss of quality by maintaining the pH within a desirable range.

In terms of titratable acidity, the addition of encapsulated bacteriocin also had a significant effect on acid production, depending on its concentration (Fig. 4). The influence of bacteriocin was effective only after 15 hr of fermentation for yoghurt containing 100 AU/mL encapsulated bacteriocin. When 500 AU/mL of bacteriocin was added, a significant difference was found after only 2 hr of fermentation.

Changes in the viability of lactic acid bacteria during fermentation The number of viable lactic acid bacterial cells during yoghurt fermentation is shown in Fig. 5. During fermentation, more bacterial cells were detected in yoghurt without bacteriocin than with bacteriocin. In control yoghurt, the growth of bacterial cells was very rapid and leveled off within 5 hr of fermentation. The two yoghurts containing bacteriocin had relatively slow, gradual increases, although rapid growth of bacterial cells occurred after 3 hr. Unlike the control yoghurt, the highest number of viable cells was observed after 15 hr of fermentation. After 5 hr of fermentation, the log values of the numbers of viable cells in the control and the 2 yoghurt samples supplemented with 100 and 500 AU/mL of bacteriocin were 9.3, 8.3, and 7.6 CFU/mL, respectively ($p<0.05$). Significant differences in viable cells were no longer observed among all 3 tested samples after 15 hr of fermentation, regardless of the presence and concentra-tion of encapsulated bacteriocin.

A change in the number of viable cells due to the addition of encapsulated bacteriocin is a major issue since

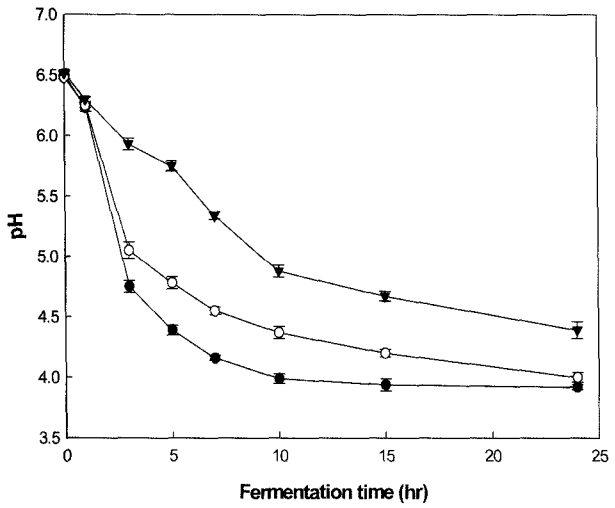

Fig. 3. Effect of encapsulated bacteriocin on the pH of yoghurts during fermentation at 42°C. (●) Control, (○) 100 AU/mL, (▲) 500 AU/mL.

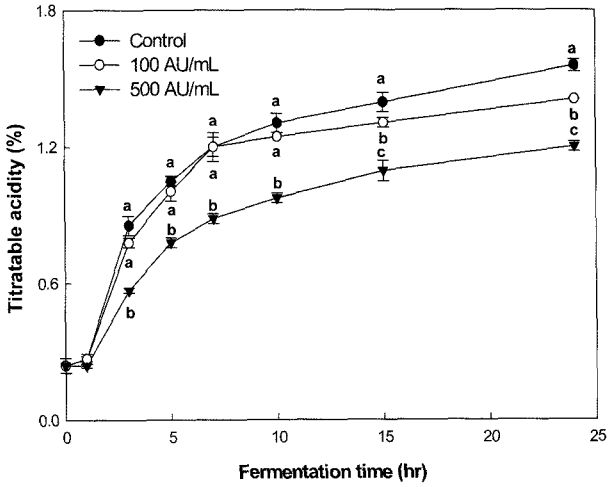

Fig. 4. Effect of encapsulated bacteriocin on titratable acidity during the fermentation of yoghurt at 42°C. (●) Control, (○) 100 AU/mL, (▲) 500 AU/mL. The different letters indicate significant differences ($p<0.05$) within the same fermentation time.

the number of viable microorganisms in the final product must satisfy regulations. Although there are some variations, the minimum number of viable bacterial cells in yoghurt required at the time of consumption is more than 10^7-10^8/mL (23). In this study, the viable microbial numbers did not show significant differences among treatments at the end of fermentation (24 hr) and were higher than log 8.5 CFU/mL.

Changes in pH and titratable acidity during cold storage at 4°C The effects of encapsulated bacteriocin on the pH and titratable acidity of yoghurts during subsequent refrigerated storage after fermentation to about pH 4.5 are shown in Fig. 5 and 6, respectively. The pH of the two yoghurts containing encapsulated bacteriocin decreased to a limited extent throughout cold storage. In contrast, the pH of the control yoghurt declined continuously throughout storage: the average pH of 4.45 before cold storage reached 4.20 at 30 days of storage. Significant differences in pH due to the presence of bacteriocin, however, were noticed only when yoghurts were stored for longer than 15 days ($p<0.05$).

The titratable acidity of the yoghurts also remained constant during cold storage in the presence of 100 and 500 AU/mL encapsulated bacteriocin, while it increased slightly in the control. There were no significant differences in titratable acidity among yoghurts until 15 days of storage. However, the titratable acidity of control yoghurt was significantly higher than that of yoghurt containing encapsulated bacteriocin when stored for longer than 20 days ($p<0.05$).

No studies have been reported with regard to the potential of encapsulated bacteriocin for controlling yoghurt fermentation through pH-dependent release. This report is the first description of using encapsulated bacteriocin in yoghurt for controlling pH and the acidification of yoghurt during fermentation. The results of this study, therefore, suggest that encapsulated bacteriocin could be effectively used to control pH and acid production in yoghurt during fermentation, and post acidification during refrigerated storage without influencing the number of viable cells. Further studies are needed to investigate sensory properties, including flavor and texture, that might be affected by prolonged fermentation time resulting from the addition of encapsulated bacteriocin.

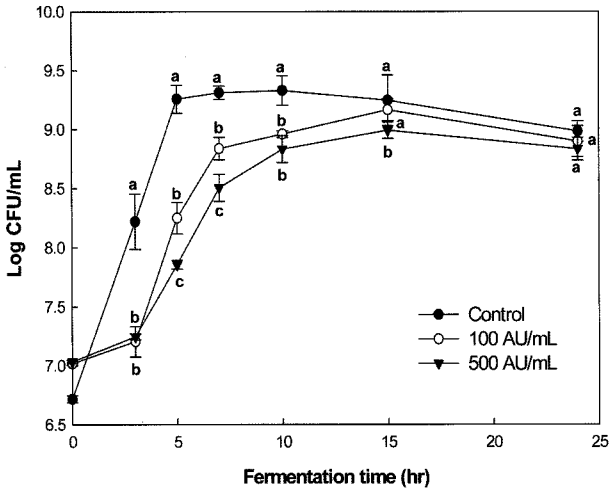

Fig. 5. Effect of encapsulated bacteriocin on the growth of lactic acid bacteria during the fermentation of yoghurt at 42 °C. (●) Control, (○) 100 AU/mL, (▲) 500 AU/mL. The different letters indicate significant differences ($p<0.05$) within the same fermentation time.

Acknowledgments

This work was supported by a grant program from Kookmin University in 2005. The authors thank for financial support.

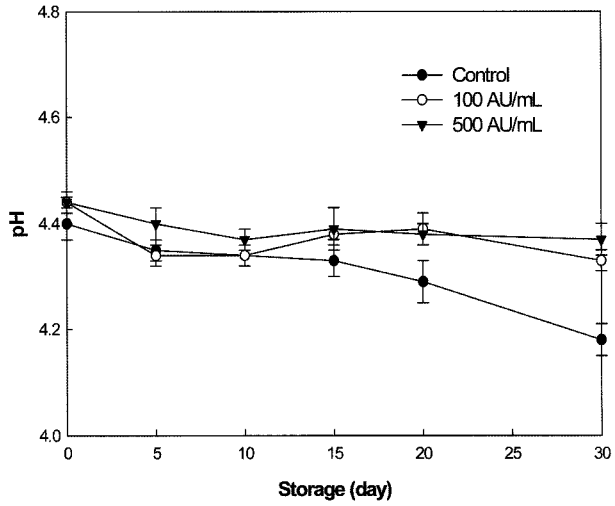

Fig. 6. Changes in the pH of yoghurt during storage at 4°C. (●) Control, (○) 100 AU/mL, (▲) 500 AU/mL.

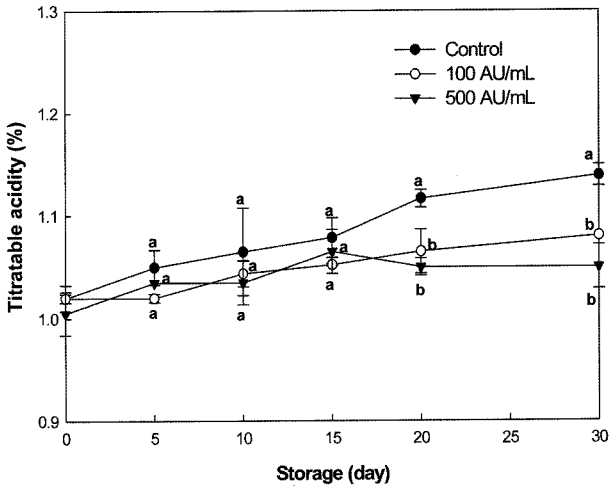

Fig. 7. Changes in the titratable acidity of yoghurt during storage at 4°C. (●) Control, (○) 100 AU/mL, (▲) 500 AU/mL. The different letters indicate significant differences ($p<0.05$) within the same storage time.

References

1. Lourens-Hattingh A, Viljeon CB. Yoghurt as probiotic carrier food. Int. Dairy J. 11: 1-17 (2001)
2. Rašić JLJ, Kurmann JA. Cultures and starters. pp. 186-213. In: Yoghurt. Rašiæ JLJ, Kurmann JA (eds). Technical Dairy Publishing House, Copenhagen, Denmark (1978)
3. Kosikowski F, Mistry VV. Yogurt. pp. 87-108. In: Cheese and Fermented Milk Foods. Virginia FV, Kosikowski LLC (eds). Kosikowski L. L. C., Great Falls, VA, USA (1999)
4. Lankaputhra WEV, Shah NP. Survival of *Lactobacillus acidophilus* and *Bifidobacterium* species in the presence of acid and bile salts. Cult. Dairy Products J. 30: 113-118 (1995)
5. Kneifel W, Jaros D, Erhard F. Microflora and acidification properties of yoghurt and yoghurt-related products fermented with commercially available starter cultures. Int. Dairy J. 18: 179-189 (1993)
6. Bake KL. Rational manufacture of stirred yogurt of improved storage life. Milchwissenschaft 26: 536-539 (1972)
7. Allen D. Quality control- milk. Ingredient, product, starter cultures. J. Soc. Dairy Technol. 48: 61-64 (1995)
8. Weinbrenner DR, Barefoot SF, Grinstead DA. Inhibition of yogurt starter cultures by jenseniin G, a *Propionibacterium* bacteriocin. J. Dairy Sci. 80: 1246-1253 (1997)
9. Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. 12: 39-86 (1993)
10. Lim SM, Park MY, Chang DS. Characterization of bacteriocin produced by *Enterococcus faecium* MJ-14 isolated from *meju*. Food Sci. Biotechnol. 14: 49-57 (2005)
11. Lee NK, Kim KT, Kim CJ, Paik HD. Optimized production of Lacticin NK24, a bacteriocin produced by *Lactococcus lactis* NK24 isolated from *jeotgal*. Food Sci. Biotechnol. 13: 6-10 (2004)
12. Joo KS, Oh S, Han KS, Jeon WM, Kim SH. Charcacterization and inhibitory activity on *Staphylococcus aureus* of a bacteriocin produced by *Lactobacillus plantarum* KU 107. Korean J. Food Sci. Anim. Resour. 22: 81-86 (2002)
13. Oh, S, Churey JJ, Worobo RW. Inhibitory activity of *Alicyclobacillus* strains by bacteriocin of *Lactococcus* sp. CU216. (abstract no 37D-32). In: Abstracts: Annual Meeting of Institute of Food Technologists, July 24-28, Chicago, IL, USA (1999).
14. Oh S, Kim SH, Worobo RW. Characterization and purification of a bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus* 30SC. J. Dairy Sci. 83: 2747-2752 (2000)
15. Han KS, Imm JY, Oh S, Jeon WM, Kim SH. Bacteriocin produced by *Lactobacillus acidophilus* ATCC 4356: Characterization and purification. Food Sci. Biotechnol. 11: 531-536 (2002)
16. Mayr-Harting A, Hedges AJ, Berkeley RCW. Methods for studying bacteriocins. Method Microbiol. 74: 315-321 (1972)
17. Ishizaka T, Honda H, Kikuchi Y, Ono K, Katano T, Koishi M. Preparation of drug-diluent hybrid powders by dry processing. J. Pharm. Pharmacol. 41: 361-368 (1989)
18. AOAC. Official Methods of Analysis of AOAC Intl. 16th ed. Method 947.05. Association of Official Analytical Chemists, Arlington, VA, USA (1995)
19. Park DJ, Imm JY, Ku KH. Improved dispersibility of green tea powder by microparticulation and formulation. J. Food Sci. 66: 793-798 (2001)
20. SAS Institute, Inc. SAS User's Guide. Ver. 6.12. Statistical Analysis Systems Institute, Cary, NC, USA (1995)
21. Ohta M, Buckton G. The use of inverse gas chromatography to asses the acid-base contributions to surface energies of cefditoren pivoxil and methacrylate copolymers and possible links to instability. Int. J. Pharm. 272: 121-128 (2004)
22. Østlie HM, Treimo J, Narvhus JA. Effect of temperature on growth and metabolism of probiotic bacteria in milk. Int. Dairy J. 15: 989-997 (2005)
23. Orihara O, Sakauchi I, Nakazawa Y. Types and standards for fermented milk and lactic drinks. pp. 1-16. In: Functions of Fermented Milk, Challenges for the Health Science. Nakazawa Y, Hosono A (eds). Elsevier Applied Science, London, UK (1992)