J. Microbiol. Biotechnol. (2006), **16**(6), 939–945

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Function of Cell-Bound and Released Exopolysaccharides Produced by *Lactobacillus rhamnosus* ATCC 9595

KIM, JI-UK[1], YOUNGHOON KIM[1], KYOUNG-SIK HAN[1], SEJONG OH[3], KWANG-YOUN WHANG[2], JAI-NEUNG KIM[4], AND SAE-HUN KIM[1]*

[1]*Division of Food Science and* [2]*Bioscience & Technology, Korea University, Seoul 136-701, Korea*
[3]*Institute of Agricultural Science & Technology, Department of Animal Science, Chonnam National University, Gwangju 500-757, Korea*
[4]*Department of Packaging, Yonsei University, Wonju 220-710, Korea*

Received: November 16, 2005
Accepted: February 18, 2006

Abstract The physiological characteristics and function of the exopolysaccharide (EPS) produced by *Lactobacillus rhamnosus* ATCC 9595 were determined. The total quantity of EPS was rapidly increased to 496±20 mg/l during the exponential phase, and then maintained steadily during the stationary phase. During the exponential phase (18 h), the total EPS consisted of 61% cell-bound EPS (cb-EPS) and 39% released EPS (r-EPS), whereas the relative proportion of EPS during the stationary phase (48 h) was converted to 23% cb-EPS and 77% r-EPS. On gel permeation chromatography, cb-EPS was fractionated as a single peak of 8.6×10^6 Da, whereas r-EPS was fractionated as two peaks with average molecular weights of 4.3×10^4 and 8.6×10^6 Da. Interestingly, both EPS species exhibited anticancer properties and cholera toxin-binding activities. Our results suggest that the EPS generated by *L. rhamnosus* ATCC 9595 might be suitable for use as a functional food or food supplement.

Key words: *Lactobacillus rhamnosus*, exopolysaccharide, cell-bound EPS, released EPS, anticancer activity, toxin-binding activity

Lactic acid bacteria (LAB), a diverse group of economically important microorganisms, are currently utilized in various foods and agricultural fermentation processes [12–14, 17, 26]. Exopolysaccharide (EPS), one of the primary metabolic products of LAB, has been receiving an increasing amount of attention in recent years [4]. EPS has thickening properties, and also appears to improve both the texture and the mouthfeel of several dairy products [19]. Many kinds of EPS, exhibiting different composition, size, and structure, are synthesized by different LAB species. In general, the EPS generated by LAB species appears to possess quite high molecular masses, ranging from 4.0×10^4 to 6.0×10^6 [11]. With these high molecular masses as well as characteristic long-chain polymers that dissolve or disperse in water to provide thickening or gelling effects, EPSs are indispensable tools in the formulation of food products. Such food polymers can also be used for the elicitation of secondary effects, including emulsification, stabilization, and suspension of particulates, crystallization control, inhibition of syneresis (the release of water from processed foods), encapsulation, and formation of films [3, 26].

LAB have been shown to generate EPS in two distinct forms: ropy EPS, which refers to a loose slime that is excreted into surrounding areas, and capsular EPS, which refers to EPS that remains adherent to the cell surface, resulting in the formation of a discrete covering [2]. Many capsular bacterial strains have been proven to produce loose slime in addition to capsular EPS [2, 3]. The term EPS is generally used to designate all forms of bacterial polysaccharides that are found outside the cell wall, and the functions of EPS have not yet been clearly defined. Capsular EPS has been suggested to perform a function in the protection of the cell from the immediate environment under unfavorable conditions [3]. In addition to their possible function as alternative biothickeners, EPS in their natural environments are believed to protect microbial cells from desiccation, phagocytosis, and phage attack antibiotics or toxic compounds, as well as predation by protozoans, osmotic stress, adhesion to solid surfaces, and biofilm formation. They have also been suggested to play a role in cellular recognition [26].

Lactobacillus rhamnosus are members of the *L. casei* group, and have recently become important as adjunct cultures for the production of fermented milks. However, few studies have been performed on EPS production by these strains, including their comparison to other LAB.

*Corresponding author
Phone: 82-2-3290-3055; Fax: 82-2-3290-3506;
E-mail: saehkim@korea.ac.kr

Interestingly, *L. rhamnosus* 9595M was reported as one of the LAB that produces high levels of EPS [8].

The objective of the present study was to characterize the production of two distinct types of EPS by *L. rhamnosus* ATCC 9595 (reference cultures for *L. rhamnosus* 9595M [8]), and to determine their effects as both anticancer and toxin-binding agents.

MATERIALS AND METHODS

Bacterial Strains, Media, and Culture Conditions for EPS Production

The EPS-producing strain, *Lactobacillus rhamnosus* ATCC 9595, was purchased from the American Type Cultue Collection (Rockville, USA). Stock cultures were stored at −80°C in 10% reconstituted skim milk (RSM) containing 20% (v/v) glycerol. Prior to use, the bacteria were subcultured twice in RSM at 37°C for 18 h. For high production of EPS, batch fermentation was carried out for 48 h in a laboratory fermentor (Biostat C; B. Braun Biotech International, Germany).

Fermentation was inoculated at 1% (v/v) of fresh culture of *L. rhamnosus* ATCC 9595 and was controlled with a pH controller (the pH was maintained at 5.5 by the addition of 5 N NaOH; Whatman Lab Sales, U.S.A.). The samples were then collected at 6-h intervals for 48 h. The EPS was quantified using the phenol-sulfuric method [7] and was expressed as a glucose standard. Cell growth was also evaluated by the number of viable cells after anaerobic incubation (BBL Gas-Pak system, Sparks, MD, U.S.A.) at 37°C for 72 h in MRS agar (Difco, MI, U.S.A.).

Apparent and Intrinsic Viscosities of EPS

Apparent viscosity measurements were conducted using a coaxial cylinder viscometer (DV-; Brookfield, U.S.A.) at a steady shear rate of 173 s^{-1}, using MK 50 rotor assembly and an NV sensor system operating at 25°C. The apparent viscosity was expressed in mPa.s. Intrinsic viscosity determinations were conducted at room temperature, using a multi-angle laser light-scattering detector (Wyatt, U.S.A.) operated at 41°, 90°, and 138° angles, a differential refractometer (ERC-7517A, dn/dc=0.146 ml/g), a UV detector (280 nm), and a differential viscometer (T-50A, Viscotek). Intrinsic viscosity was expressed in dl/g.

Isolation and Purification of Two Types of EPS

Extraction of Released EPS (r-EPS). The extraction of r-EPS was performed according to the method of Zisu and Shah [28]. The crude EPS was collected by centrifugation (14,000 ×*g*, 30 min, 4°C) and resuspended in 0.05 M Tris-HCl buffer (pH 8.0). The contaminating proteins were digested overnight with 0.2 mg/ml of proteinase K (Sigma, U.S.A.), at 37°C. The reaction was stopped by heat treatment (90°C for 10 min), and the EPS was precipitated from solution with 15 ml of cold ethanol, followed by centrifugation. The EPS pellet was then suspended in sterile distilled water, and sonicated for 120 min in order to soften it.

Extraction of Cell-Bound EPS (cb-EPS). The cb-EPS extraction method used in this study was adapted from the method described by Toba *et al.* [24]. The EPS was precipitated from the supernatant by the addition of two volumes of cold ethanol, followed by incubation overnight at 4°C. After centrifugation, the pellet that contained the cb-EPS was resuspended in distilled water and dialyzed (molecular weight cutoff: 6,000–8,000 Da) against 2 l of distilled water for 2 days, with three water changes per day.

Determination of Molecular Mass

The molecular masses of the two isolated EPS fractions were then estimated by gel permeation chromatography (Sephacryl S-500 column; Pharmacia, Sweden). A standard dextran series (molecular masses of 2.7×10^4, 4.3×10^4, 6.7×10^5, 7.5×10^5, 1.0×10^6, and 8.5×10^6) was used. Ammonium hydrogen carbonate (0.05 M) was used as an elution buffer, at a flow rate of 2 ml/min. All of the collected fractions were assessed for EPS content.

Monosaccharide Composition of Isolated EPS

In order to assess its monosaccharide composition, EPS was hydrolyzed with 4 N trifluoroacetic acid (TFA) at 120°C for 150 min. After alkalinization, unhydrolyzed EPS was eliminated via centrifugation before sugars were converted to alditol acetate. The content of alditol acetate was then determined via gas liquid chromatography, again using inositol as an internal standard [23].

Cell Culture

Two human cancer cell lines and one normal cell line were utilized in the verification of anticancer activity exhibited by the EPS. All media and fetal bovine serum (FBS) were obtained from Gibco BRL (Life Technologies Inc., Grand Island, NY, U.S.A.) and JBI (Jeil Biotechservices Inc., Seoul, Korea). PANC-1 (pancreas) and HT-29 (colon) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 medium that had been supplemented with 10% FBS, respectively. The cells were cultured routinely at 37°C in a humidified atmosphere with 5% CO_2.

Determination of Inhibition of Cancer Cell Growth

The MTT assay is based on the reduction of tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), by actively growing cells. Two cancer cell lines were seeded in a 96-well plate (1.0×10^4 cells per well). After formation of monolayer, b-EPS and r-EPS were dissolved in corresponding media without FBS at 0.1, 0.5, 1, and 5 mg/ml concentrations. The cells in EPS

containing media were cultured for 72 h. Fifty μl of the 2.0 mg/ml stock solution of MTT was added to each well. After 4 h of incubation, the culture media were removed, 100 μl of dimethly sulfoxide (DMSO) was added to each well for dissolving the formazan dye, and dissolved dye was quantified at 550 nm using an ELISA reader (Molecular Device, Sunnyvale, CA, U.S.A.).

Using an ApoProbe-3 kit (Peptron Inc., Daejun, Korea) to ascertain apoptosis, the caspase-3 fluorescent assay was performed according to the manufacturer's instructions with a fluorometer (360 nm excitation and 460 nm emission filter).

Cholera Toxin-Binding Activity

Cholera toxin-binding activity was also performed as previously described [21] with slight modification. Briefly, two freeze-dried EPSs were dissolved in PBS buffer to 5 mg/ml, respectively. Cholera toxin B subunit (Sigma, U.S.A.) was diluted with the ratio of 1:100, 1:200, 1:400, 1:800, 1:1,600, 1:3,200, 1:6,400, and 1:12,800. Thirty μl of diluted cholera toxin was mixed with each EPS solution at 37°C for 2 h and washed three times with PBS buffer. Twenty milliliters of OPD substrate solution (5 mg *o*-phenylenediamine dihydrochloride-carbonate buffer-0.02% H_2O_2) was added, and the mixture was incubated at 37°C for 30 min. Twenty liter of 0.5 M H_2SO_4 was added to terminate the reaction, and the plate was read at 490 nm using an ELISA reader.

Statistical Analysis

All of the experiments conducted in this study were repeated three times. Statistical analyses were performed by SAS using the general linear model procedures. The level of significance was defined at $p<0.05$ using Tukey's test.

Results and Discussion

Production of EPS by *L. rhamnosus* ATCC 9595

Results on the localization of EPS in *L. rhamnosus* ATCC 9595 grown at 37°C in RSM are presented in Fig. 1. The total amount of EPS (sum of cell-bound and released EPS fractions) increased rapidly up to 496±20 mg/l as the cell number increased during the exponential phase, and remained at a steady level during the stationary phase. The contents of EPS produced by LAB cultures were generally very low. The amount of EPS has been reported to vary from 25 to 132 mg/l for *Lactococcus latis* subsp. *cremoris* [18] and *L. rhamnosus* C83 [9], and from 130 to 250 mg/l for *L. casei* CG11 [4] and *L. delbreuckii* subsp. *bulgaricus* NCFB 2772 [10], respectively. Therefore, *L. rhamnosus* ATCC 9595 should be classified among the strains with a best maximum EPS production of 496±20 mg/l.

The proportion of cb-EPS increased to 61% of the total amount of EPS, whereas r-EPS comprised 39% fraction

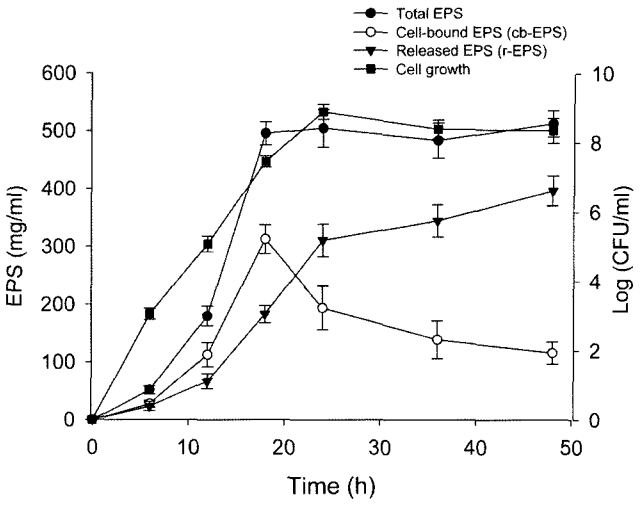

Fig. 1. EPS production and cell growth of *L. rhamnosus* ATCC 9595 during 48 h of fermentation. Values are expressed as means ±SE.

until the exponential phase. However, the quantity of cb-EPS gradually decreased from the beginning of the stationary phase, and reduced to 23% of the total EPS after 48 h of fermentation. Marshall *et al.* [18] reported that *Lactococcus lactis* subsp. *cremoris* LC330 was capable of producing two polysaccharides that are different in composition and molecular mass: One polymer was closely associated with the cell wall materials, and the other was secreted into the culture medium. In the present study, the reduction of cb-EPS content in the stationary phase might have been due to desorption of the polymer rather than enzymatic degradation, since the glycohydrolases produced by *L. rhamnosus* are reported to have no effects on the molecular mass of the polymer [22]. The progressive release of the cb-EPS fraction might explain the continuous increase of r-EPS observed during the stationary growth phase, which is closely coupled with a concomitant reduction of cb-EPS. Fermentation assays, which were carried out under our defined conditions, suggest that EPS production may be closely associated with cell growth: The yield of EPS increased during the exponential growth phase, and no further production was observed throughout the stationary phase [9].

The total yield of EPS produced by LAB depends on the composition of the medium (carbon and nitrogen sources) as well as conditions in which the strains grow; *i.e.*, temperature, pH, and incubation time [8]. Our unpublished data showed that the r-EPS produced by *L. rhamnosus* ATCC 9595 in RSM supplemented with 1% WPC significantly increased to 1,011±14.7 mg/l (Kim and Kim, unpublished data). We are presently investigating optimal conditions for industrial scale production of EPS, using the response surface method (RSM).

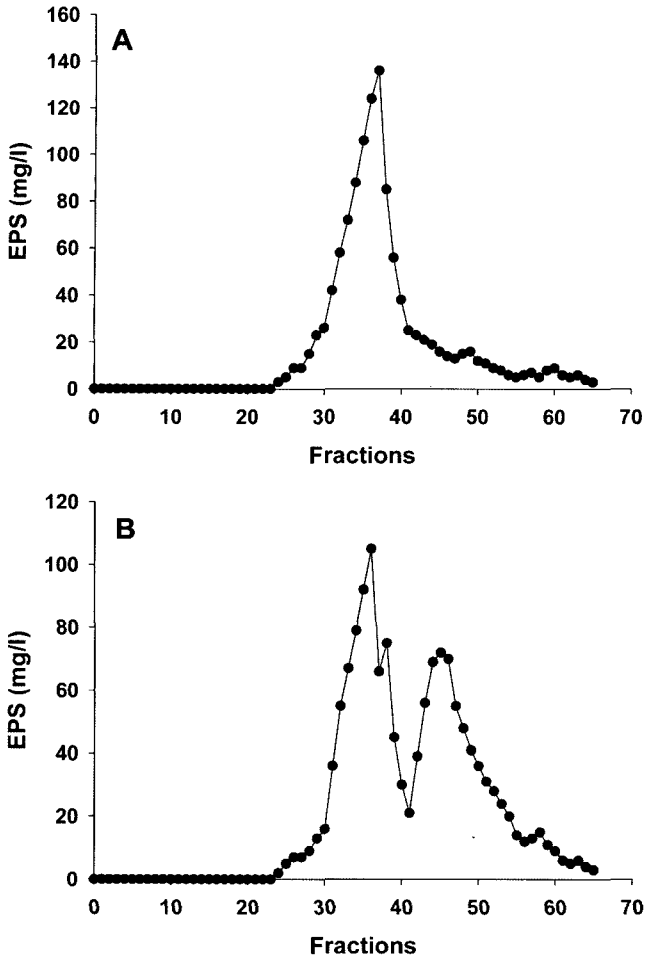

Fig. 2. Gel permeation chromatogram of cell-bound EPS (cb-EPS; A) and released EPS (r-EPS; B) produced by *L. rhamnosus*. The molecular mass of EPS was estimated via gel permeation chromatography (Sephacryl S-500 column; Pharmacia, Uppsala, Sweden). Ammonium hydrogen carbonate (0.05 M) was used as an elution buffer at a flow rate of 2 ml/min.

Determination of Molecular Weights of Two Types of EPS

Two types of EPS, designated as r-EPS and cb-EPS, were produced by *L. rhamnosus* ATCC 9595 in RSM at 37°C. Figure 2A shows the gel permeation chromatography elution profiles and size distribution analysis of cb-EPS synthesized by *L. rhamnosus* ATCC 9595. The cb-EPS of this strain showed only one peak, which corresponded to a molecular mass of 8.6×10^6 Da. However, the r-EPS was resolved into two peaks, corresponding to molecular masses of 4.3×10^4 and 8.6×10^6 Da, respectively (Fig. 2B). The isolation of both fractions from the two different EPS variants indicated similar elution patterns with regard to the high molecular mass of EPS. Similarly, Degeest and de Vuyst [5] also isolated two EPS fractions from the culture of *S. thermophilus* LY03, one with a high (1.8×10^6 Da) and the other with a low (1.4×10^5 Da) molecular mass. Indeed, many studies isolated two types of LAB-generated EPS, which exhibited molecular masses of 10^4–10^6 Da [11, 18]. Therefore, the production of two EPS fractions may be a general feature of LAB under fermentation conditions, including *L. rhamnosus*.

Monosaccharide Composition of EPS

Table 1 presents the viscosity and relative monosaccharide compositions of each of the EPSs produced by *L. rhamsosus* ATCC 9595. Both the high molecular EPS and cb-EPS exhibited similar viscosity values and monosaccharide compositions. Moreover, they had similar elution profiles and molecular masses. Galactose and glucose were shown to be the major constituents of the EPS produced by *L. rhamnosus* ATCC 9595: Galactose content was 32–62% and glucose content was 31.2–62.7%. Landersjö *et al.* [16] reported that galactose and glucose constituted the primary sugars in the EPS isolated from *L. rhamnosus* GG. Unfortunately, there was no description on cb-EPS produced by *L. rhamnosus*.

The cb-EPS and high molecular EPS produced by *L. rhamnosus* ATCC 9595 exhibited similar viscosity, and r-EPS and low molecular EPS were determined to have more rhamnose than cb-EPS and high molecular EPS did, whereas the glucose content of r-EPS and low molecular EPS was found to be lower than that of cb-EPS and high molecular EPS. It appears that cb-EPS consists of high molecular EPS, and that high molecular EPS is associated with significant viscosity. In addition, there was no significant difference in the viscosity between EPS isolated during the exponential and stationary phases (data not shown).

Table 1. Viscosity and monosaccharide composition of EPS produced by *Lactobacillus rhamosus* ATCC 9595.

EPS fraction[1]	Apparent viscosity[2] (mPa.s)	Intrinsic viscosity[2] (dl/g)	Monosaccharide			
			Galactose	Glucose	Rhamnose	Mannose
cb-EPS	411.6±17.4[a]	9.3±0.9[a]	32.0	61.5	1.4	5.1
r-EPS	297.4±15.5[b]	6.5±1.3[b]	50.6	31.2	15.7	2.5
High-molecular	422.3±18.6[a]	9.8±1.1[a]	32.9	62.7	1.1	3.3
Low-molecular	46.3±5.6[c]	1.9±0.2[c]	63.8	21.6	13.5	1.1

[1]b-EPS, r-EPS, high-molecular, and low-molecular signify cell-bound EPS, released EPS, high and low molar mass EPS fractions, respectively.
[2]Each value is expressed as mean±SE.
[a,b,c]Different letter superscripts within the same column indicate differences at $p<0.05$.

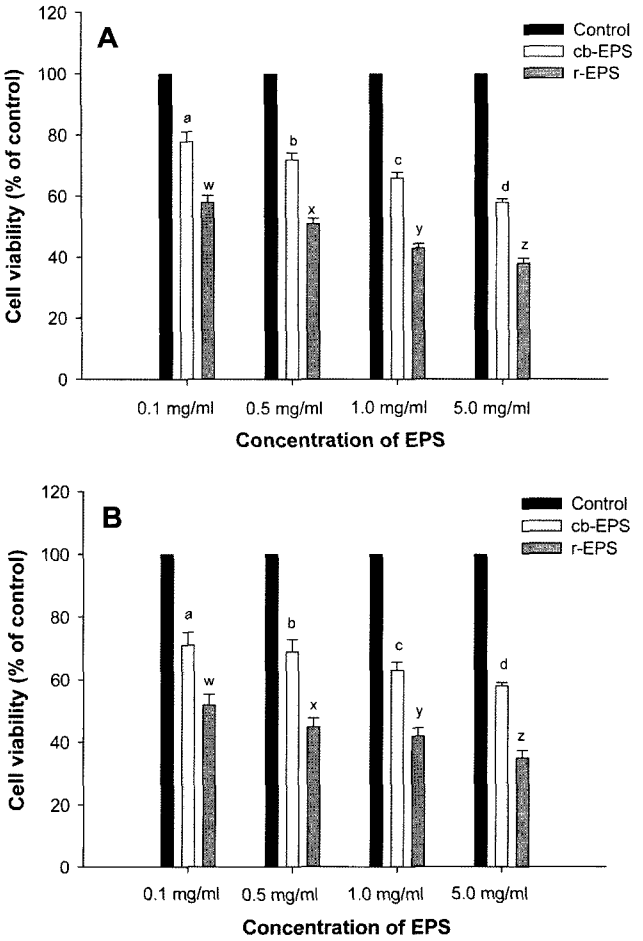

Fig. 3. Anticancer activities of cb-EPS and r-EPS against the PANC-1 (**A**) and HT-29 (**B**) cell lines, evidenced by MTT assay. Cells were cultured for 72 h on media treated with different EPSs. $^{a-d}$Means with different letter superscripts indicate differences with p values of <0.05 within cells on media to which cb-EPS has been added. $^{w-z}$Means with different letter superscripts indicate differences with p values of <0.05 within cells on media to which r-EPS has been applied.

Anticancer and Cholera Toxin-Binding Activities by EPS

Until now, there have been few reports on the functional activities of EPS produced by various LAB including *L. rhamnosus*. Therefore, in the present study, functions such as anticancer and toxin-binding activities were investigated with EPSs isolated from *L. rhamnosus* ATCC 9595.

The inhibitory effects of cb-EPS and r-EPS against two cancer cell lines used in this study are shown in Fig. 3. At all concentrations tested, EPSs were shown to suppress the growth of PANC-1 and HT-29 cells for 72 h. In particular, at 5 mg/ml concentration, they were found to significantly inhibit the growth of both cancer cell lines. However, they had no effects on the normal cell line, hEF (data not shown). Interestingly, r-EPS was more effective in anticancer activity than cb-EPS. In analogous experiments, no significant effects were observed on cell proliferation of HT-29, when

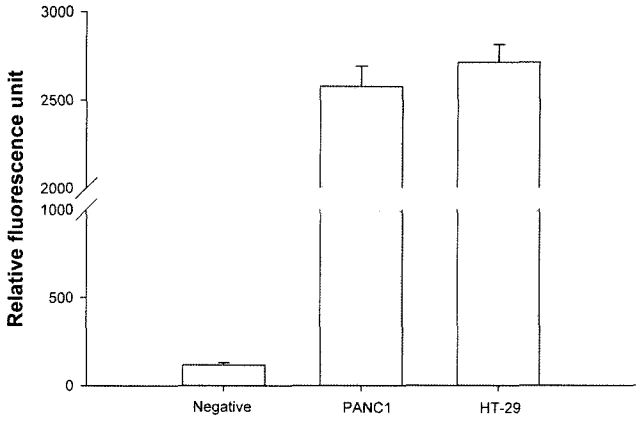

Fig. 4. Alteration of caspase-3 activity in PANC1 and HT-29 cells by r-EPS (5.0 mg/ml).

only rhamnose was added (0.5, 1, and 2 mg/ml; data not shown). Experimental studies indicate that LAB might decrease the risk of certain type of cancers and inhibit the growth and viability of the human colon cancer cell line [1, 20]. However, the precise mechanisms by which EPS exerts anticancer effects remains unknown. Several researchers [25, 27] reported that biological activity, such as anticancer, of polysaccharide isolated from natural materials was influenced by their solubility in water, molecular weight, and branching rate and type. Therefore, it is highly likely that the anticancer activities of EPSs in this study were due to structural factors such as polysaccharide branching types, but not monosaccharide compositions.

When treated with 5 mg/ml of EPS, the production of caspase-3 in both the PANC-1 and HT-29 cells was remarkably increased, compared with the control (Fig. 4). Therefore, these results strongly suggest that the inhibitory effect of the EPS on cancer cells might be attributable, at least in part, to the induction of apoptosis. We are in a process to evaluate the apoptosis-inducing pathway by the EPSs isolated from *L. rhamnosus* ATCC 9595, using proteomic works.

The two types of EPSs were assayed for binding activity to the cholera toxin B-subunit. As shown in Fig. 5, both EPSs were shown to inhibit toxin-binding ability: The r-EPS exhibited a stronger effect against cholera toxin-binding than cb-EPS, indicating that EPS is able to inhibit the reaction between cholera toxin B and epithelium cells.

To date there have been only a few reports on the inhibition of toxin activity by natural materials such as EPS. Some studies showed that culture supernatant of *Bifidobacterium* and glycomacropeptide (GMP) isolated from k-casein, as toxin-neutralizing components, were able to inhibit the cytotoxicity of shiga-like toxin and cholera toxin, respectiverly [13, 21]. To our best knowledge, however, this study is the first report on the toxin-binding activity of EPSs produced by LAB.

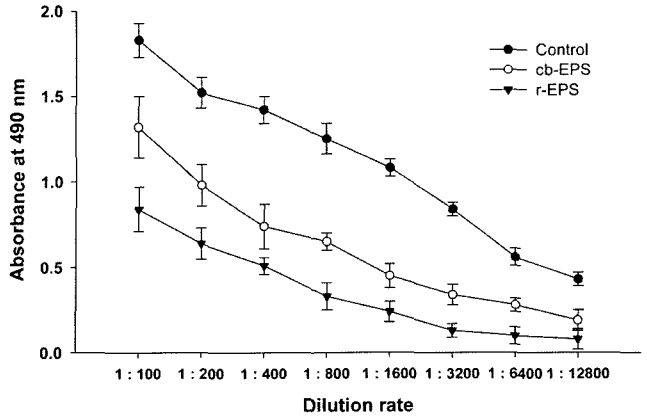

Fig. 5. The binding activity of cb-EPS and r-EPS to the cholera toxin B subunit (filled circles, control; blank circles, cb-EPS with 5.0 mg/ml; and filled triangles, r-EPS with 5.0 mg/ml).

In conclusion, the two EPS variants derived from *L. rhamnosus* ATCC 9595 were successfully purified in this study, and their physicochemical properties were studied. Both types of EPS were also found to exhibit the profound cholera toxin-binding activity, as well as anticancer activity in *in vitro* trials. These EPS components could be used in the development of a variety of natural food additives or adjuncts for either cancer therapy or prevention of cholera-associated food poisoning.

Further study is currently underway to analyze the structures of these EPS variants, using NMR spectroscopy, and to investigate the anticancer and toxin-binding activities of EPSs from *L. rhamnosus* ATCC 9595 by the killing assay of *Caenorhabditis elegans in vivo* model.

Acknowledgments

This work was supported by a grant (20050401-034-698-151-00-00) from the BioGreen 21 Program, Rural Development Administration, Republic of Korea. Y. Kim was supported by Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-F00010).

References

1. Baricault, L., G. Denariaz, J. J. Houri, C. Bouley, C. Sapin, and G. Trugnan. 1995. Use of HT-29, a cultured human colon cancer cell line, to study the effect of fermented milks on colon cancer cell growth and differentiation. *Carcinogenesis* **16:** 245–252.
2. Broadbent, J. R., D. J. Mcmahon, D. L. Welker, C. J. Oberg, and S. Moineau. 2003. Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: A review. *J. Dairy Sci.* **86:** 407–423.
3. Cerning, J. 1990. Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiol. Rev.* **87:** 113–130.
4. Cerning, J., C. M. G. C. Renard, J. F. Thibault, C. Bouillanne, M. Landon, M. Desmazeaud, and L. Topisirovic. 1994. Carbon source requirements for exopolysaccharides production by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. *Appl. Environ. Microbiol.* **60:** 3914–3919.
5. Degeest, B. and L. De Vuyst. 2000. Correlation of activities of the enzymes alpha-phosphoglucomutase, UDP-galactose 4-epimerase, and UDP-glucose pyrophosphorylase with exopolysaccharide biosynthesis by *Streptococcus thermophilus* LY03. *Appl. Environ. Microbiol.* **66:** 3519–3527.
6. De Vuyst, L., F. Vanderveken, S. Van de Ven, and B. Degeest. 1998. Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *J. Appl. Microbiol.* **84:** 1059–1068.
7. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colometric method for determination of sugar and related substances. *Anal. Chem.* **28:** 350–356.
8. Dupont, I., D. Roy, and G. Lapointe. 2000. Comparison of exopolysaccharide production by strains of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* grown in chemically defined medium and milk. *J. Ind. Microbiol. Biotechnol.* **24:** 251–255.
9. Gamar, L., K. Blondeau, and J. M. Simonet. 1997. Physiological approach to extracellular polysaccharide production by *Lactobacillus rhamnosus* strain C83. *J. Appl. Microbiol.* **83:** 281–287.
10. Grobben, G. J., J. Sikkema, M. R. Smith, and J. A. De Bont. 1995. Production of extracellular polysaccharides by *Lactobacillus delbrueckii* ssp. *bulgaricus* NCFB 2772 grown in a chemically defined medium. *J. Appl. Bacteriol.* **79:** 103–107.
11. Grobben, G. J., W. H. M. Van Casteren, H. A. Schols, A. Oosterveld, G. Sala., M. R. Smith, J. Sikkema, and J. A. M. De Bont. 1997. Analysis of the exopolysaccharides produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in continuous culture on glucose and fructose. *Appl. Microbiol. Biotechnol.* **48:** 516–521.
12. Kim, H. J., J. H. Kim, J. H. Son, H. J. Seo, S. J. Park, N. S. Paek, and S. K. Kim. 2004. Characterization of bacteriocin produced by *Lactobacillus bulgaricus*. *J. Microbiol. Biotechnol.* **14:** 503–508.
13. Kim S. H., S. J. Yang, H. C. Koo, W. K. Bae, J. Y. Kim, J. H. Park, Y. J. Baek, and Y. H. Park. 2001. Inhibitory activity of *Bifidobacterium longum* HY8001 against Vero cytotoxin of *Escherichia coli* O157:H7. *J. Food Prot.* **64:** 1667–1673.
14. Kim, S. J., J. H. Kim, J. Y. Park, H. T. Kim, S. J. Jeong, Y. L. Ha, H. D. Yun, and J. H. Kim. 2004. Cold adaptation of *Lactobacillus paraplantarum* C7 isolated from *kimchi*. *J. Microbiol. Biotechnol.* **14:** 1071–1074.
15. Kim, T. W., H. S. Song, and H. Y. Kim. 2005. Distribution of dominant Bifidobacteria in the intestinal microflora of Korean adults and seniors, identified by SDS-PAGE of whole cell proteins and 16S rDNA sequence analysis. *J. Microbiol. Biotechnol.* **15:** 388–394.

16. Landersjö, C., Z. Yang, E. Huttunen, and G. Widmalm. 2002. Structural studies of the exopolysaccharide produced by *Lactobacillus rhamnosus* strain GG (ATCC 53103). *Biomacromolecules* **3:** 880–884.
17. Lee, J. H., M. J. Kim, D. W. Jeong, M. J. Kim, J. H. Kim, H. C. Chang, D. K. Chung, H. Y. Kim, K. H. Kim, and H. J. Lee. 2005. Identification of bacteriocin-producing *Lactobacillus paraplantarum* first isolated from kimchi. *J. Microbiol. Biotechnol.* **15:** 428–433.
18. Marshall, V. M., E. N. Cowie, and R. S. Moreton. 1995. Analysis and production of two exopolysaccharides from *Lactococcus lactis* subsp. *cremoris* LC330. *J. Dairy Res.* **62:** 621–628.
19. Marshall, V. M. and H. L. Rawson. 1999. Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt. *Int. J. Food Sci. Technol.* **34:** 137–143.
20. Oda, M., H. Hasegawa, S. Komatsu, K. Kambe, and F. Tsuchiya. 1983. Antitumor polysaccharides from *Lactobacillus* subsp. *Agric. Biol. Chem.* **47:** 1623–1625.
21. Oh, S., R. W. Worobo, B. C. Kim, S. Rheem, S. H. Kim. 2000. Detection of the cholera toxin-binding activity of kappa-casein macropeptide and optimization of its production by the response surface methodology. *Biosci. Biotechnol. Biochem.* **64:** 516–522.
22. Pham, P. L., I. Dupont, D. Roy, G. Lapointe, and J. Cerning. 2000. Production of exopolysaccharide by *Lactobacillus rhamnosus* R and analysis of its enzymatic degradation during prolonged fermentation. *Appl. Environ. Microbiol.* **66:** 2302–2310.
23. Petry, S., S. Furlan, M. J. Crepeau, J. Cerning, and M. Desmazeaud. 2000. Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* grown in a chemically defined medium. *Appl. Environ. Microbiol.* **66:** 3427–3431.
24. Toba, T., T. Kotani, and S. Adachi. 1991. Capsular polysaccharide of a slime-forming *Lactococcus lactis* ssp. *cremoris* LAPT 3001 isolated from Swedish fermented milk 'langfil'. *Int. J. Food Microbiol.* **12:** 167–172.
25. Wasser, S. P. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biotechnol.* **60:** 258–274
26. Welman, A. D. and I. S. Maddox. 2003. Exopolysaccharides from lactic acid bacteria: Perspectives and challenges. *Trends Biotechnol.* **21:** 269–274.
27. Zaidman, B. Z., M. Yassin, J. Mahajna, and S. P. Wasser. 2005. Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Appl. Microbiol. Biotechnol.* **67:** 453–468.
28. Zisu, B. and N. P. Shah. 2003. Effects of pH, temperature, supplementation with whey protein concentrate, and adjunct cultures on the production of exopolysaccharides by *Streptococcus thermophilus* 1275. *J. Dairy Sci.* **86:** 3405–3415.