Biochemical and Biophysical Research Communications 379 (2009) 324–329

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Released exopolysaccharide (r-EPS) produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic *Escherichia coli* O157:H7

Younghoon Kim [a], Sejong oh [b], Sae Hun Kim [a,*]

[a] Division of Food Bioscience and Technology, Korea University, Seoul 136-701, Republic of Korea
[b] Department of Animal Science, Chonnam National University, Gwangju 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 3 December 2008
Available online 25 December 2008

Keywords:
EHEC O157:H7
Lactobacillus acidophilus
Biofilm formation
Exopolysaccharide

ABSTRACT

Here, we characterized released-exopolysaccharides (r-EPS) from *Lactobacillus acidophilus* A4 with the goal of identifying natural compounds that represses biofilm formation. In plastic 96-well microplates that contained 1.0 mg/ml of r-EPS, enterohemorrhagic *Escherichia coli* (EHEC) biofilms were dramatically decreased by 87% and 94% on polystyrene and polyvinyl chloride (PVC) surfaces, respectively. In the presence of r-EPS, neither their growth rate nor their autoinducer-2-like activity was affected on the EHEC O157:H7. Importantly, consistent reduction in biofilm formation was also observed when r-EPS was applied to the continuous-flow chamber models. In addition, we found that adding r-EPS significantly repressed biofilm formation by affecting genes related to curli production (*crl*, *csgA*, and *csgB*) and chemotaxis (*cheY*) in transcriptome analysis. Furthermore, these r-EPS could prevent biofilm formation by a wide range of Gram-negative and -positive pathogens. This property may lead to the development of novel food-grade adjuncts for microbial biofilm control.

Enterohemorrhagic *Escherichia coli* (EHEC) is a human pathogen that enters the intestinal tract as a result of food contamination and causes hemorrhagic colitis and hemolytic uremic syndrome (HUS). Recently, it was shown that pathogenic bacteria including EHEC O157:H7 can exist in two different modes of growth, the first being as single, planktonic cells and the second as a structured, multi-cellular consortium known as biofilms [1,2]. Compared with free-swimming micro-organisms, bacterial growth on surfaces induces novel behaviors such as an increased tolerance to stress, biocides, and host immunological defenses [3]. Therefore, biofilms formed by potentially pathogenic bacteria are considered an important cause of chronic and recurrent infections, particularly because of their capacity to form and persist on medical surfaces and indwelling devices [4]. Recently, there has been a tremendous increase in biofilm research, mostly with the ultimate aim of biofilm prevention, control, or eradication [5]. Meanwhile, only a few studies have focused on identifying and characterizing natural compounds that inhibit biofilm formation by *E. coli*, including the EHEC strains, while not affecting cell growth. The compounds (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone from the marine alga *Delisea pulchra* [6] and ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) from the ebony tree *Diospyros dendo* from Gabon [7], and group II capsular polysaccharides produced by nosocomial *E. coli* [4] significantly inhibit biofilm formation by *E. coli* without inhibiting its growth. However, because of their toxicity and low solubility in water, the use of these chemicals in the food industry has been limited.

Lactic acid bacteria (LAB) including *Lactobacillus acidophilus* possess functions that are of importance to the health and well being of the host cells [2]. It has been well established that these bacteria may represent effective tools for controlling the overgrowth of pathogens and thereby controlling or preventing infections [8,9]. Exopolysaccharides (EPS), one of the primary metabolic products of LAB, have received an increasing amount of attention in recent years, and EPS have been attributed to positive health effects [10]. However, the functional role that EPS plays in bacterial ecology still remains uncertain. Moreover, a recent study reported that a number of EPS isolated from commercial fermented milk "villi" were capable of interfering with the adhesion of several enteric pathogens [11]. However, to date, there have been only a few reports regarding the inhibition of biofilm formation by EPS produced by probiotic bacteria. Therefore, our objective was to characterize the biofilm inhibitory properties of released type EPS (r-EPS) produced by *Lactobacillus* strains in 96-well surfaces and continuous-flow chamber biofilm models.

Materials and methods

Bacterial strains, cell lines, and cultivation

Lactobacillus acidophilus A4 was cultured in Man, Rogosa Sharpe (MRS) broth (Difco, Detroit, MI, USA) at 37 °C for 18 h. Prior to experimental use, the bacteria were sub-cultured twice in 10%

* Corresponding author. Fax: +82 2 3290 3506.
E-mail address: saehkim@korea.ac.kr (S.H. Kim).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.12.053

reconstituted skim milk (RSM) at 37 °C for 18 h. Pathogenic bacteria including EHEC O157:H7, were grown at 37 °C in Luria–Butani (LB) and Tryptic Soy Broth (TSB) medium. The HT-29 cell line was acquired from the Korea Cell Line Bank (KCLB; Seoul, Korea). The cells were routinely cultured in RPMI 1640 medium (Gibco–BRL Life Technologies, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT, USA), penicillin G (100 IU/ml), and streptomycin (100 μg/ml), at 37 °C in an atmosphere containing 5% CO_2.

Isolation of functional EPS

(1) Released-EPS (r-EPS). The extraction of r-EPS was performed according to the method of Kim et al. [10], with modifications. Briefly, the crude EPS pellets, which were precipitated by ethanol, were suspended in sterile distilled water, boiled for 5 h at 70 °C, and centrifuged (14,000*g*, 30 min, 4 °C) to remove the insoluble fractions. Finally, soluble r-EPS was recovered, adjusted to pH 7.2, and re-lyophilized. The EPS was quantified using the phenol–sulfuric acid method [12], which was expressed relative to a glucose standard.

(2) Cell-bound EPS (cb-EPS). The cb-EPS extraction method used was adapted from the method described by Toba et al. [13] and Kim et al. [10]. After sonicating the cell pellets, cb-EPS was precipitated from the supernatant and the soluble fraction of cb-EPS was extracted. The same procedure described above was used for extraction of r-EPS.

Biofilm formation on a plastic surface

The biofilm formation protocol was adapted from the protocol of Kim et al. [2] with polystyrene and polyvinyl chloride (PVC) 96-well microplates (BD Biosciences, San Jose, CA, USA).

Autoaggregation and attachment on HT-29 cells

Autoaggregation assays were performed as described by Beloin et al. [14], with slight modifications. EHEC cells were grown in LB medium at 37 °C for 18 h. Attachment assays were adapted from the method described by Kim et al. [2]. EHEC O157:H7 ($\sim 10^6$ CFU/ml) grown in LB medium were cultured at 37 °C in a 5% CO_2 atmosphere for 3 h in the absence or presence of 1.0 mg/ml r-EPS. The HT-29 monolayers were then washed with PBS six times to remove unattached bacteria. The adherent cells were released from well plates using 0.1% Triton X-100 for 10 min. Attachment ability (CFU/ml) was determined by counting on MacConkey agar.

AI-2 like activity bioassay

EHEC O157:H7 supernatants with crude AI-2-like activity were assayed using a previous described method [15]. Reporter strains of *V. harveyi* BB170 (AI-2 sensor strain) were grown in AB medium overnight and diluted to 1:5000 in fresh AB medium supplemented with 1.0 mg r-EPS/ml. The time course of bioluminescence was measured using a luminometer (Wallac model 1420 multilabel counter; Perkin-Elmer, Boston, MA, USA) and expressed as relative light units (RLU).

Flow chamber biofilm assay

Biofilms were formed in glass capillary tubes under continuous-flow conditions, as described by Kim et al. [2] with slight modifications. The flow chambers, with individual channel dimensions of 1 × 4 × 40 mm (Stovall Life Sciences, Greensboro, NC, USA), were inoculated with 1 ml (*ca* 10^9 CFU/ml) of an overnight culture of EHEC grown in LB medium with or without 0.1 and 1.0 mg/ml r-

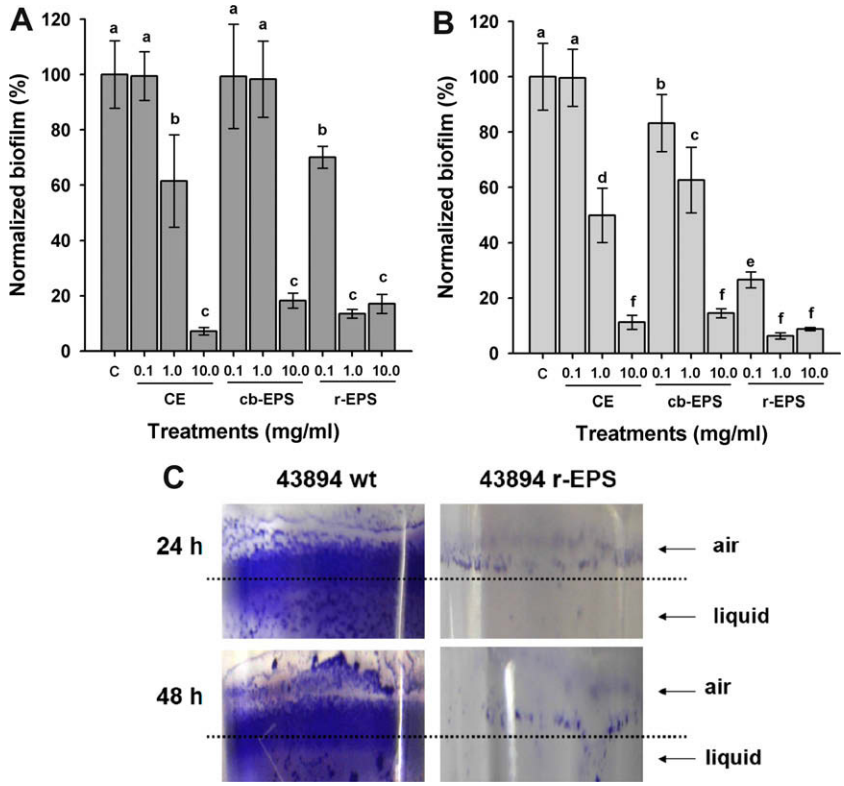

Fig. 1. Effects of EPS isolated from *L. acidophilus* A4 on biofilm formation. The quantification of biofilm formation was carried out on the 96-well polystyrene (A) and PVC (B) plates. Means and standard deviation (SD) of three independent experiments are shown. (C) Microscopic analysis of the inhibition of EHEC O157:H7 43894 biofilm formation by r-EPS. EHEC 43894 cells were incubated in PVC microtiter plates in 1/2-diluted LB medium in the presence or absence of r-EPS (1.0 mg/ml).

EPS. The inoculum was injected by a syringe to fill the inside of the glass tubes. The tubing upstream of the glass tube was clamped, and the system was allowed to stand without flow for 30 min at room temperature. After inoculation, the capillary tubes were mounted on a standard microscope slide. The system was switched to a continuous culture mode by delivering 1/2-diluted LB medium to the home-made carboy at a flow rate of 0.2 ml/min at 30 °C. The r-EPS (1.0 mg/ml) was added at the time of inoculation and to the fresh medium to investigate its inhibitory effects on EHEC biofilm formation. Biofilm development in the flow chamber was monitored using transmitted light and a 10× objective lens with an Olympus CH2 microscope (Tokyo, Japan).

Transcriptome analysis using DNA microarray

EHEC O157:H7 was grown in LB medium overnight, and an overnight culture (*ca* 10^6 CFU/ml) was inoculated in 1/2-diluted LB (a 250 ml shake flask) with or without 1.0 mg/ml r-EPS. The cells were incubated with shaking (200 rpm) at 37 °C to a turbidity of 2 at OD_{600}. Then, bacteria pellets were quickly collected by centrifugation (4 °C, 14,000*g*, 10 min) and pooled. Total RNA was isolated using the RNeasy Total RNA Extraction Kit (Qiagen, Hilden, Germany) by following the manufacturer's instructions and further purified using the acidic phenol–chloroform extraction. The purified RNA was stored at −80 °C until use.

In this study, GenePlorer TwinChip™ *E. coli*-6K (Digital Genomics, Seoul, Korea) was used for transcriptome analysis. Oligo microarray chips were hybridized with the fluorescently labeled cRNA at 60 °C for 16 h. Finally, the hybridized microarray was scanned with a GenePix 4000B dual-channel confocal laser scanner (Axon Instruments, Union City, CA, USA) at 532 nm for Cy3 and 635 nm for Cy5. Image analysis using GenePix Pro 3.0 (Axon Instruments) produced quantitative values for each microarray spot. Spot intensities were normalized by using a per-spot and per-chip intensity-dependent (Lowess) normalization method. Genes showing a 2-fold or greater difference in the expression ratio between the two conditions were classified as regulated genes ($p < 0.05$).

Results and discussion

Effects of r-EPS on EHEC biofilm formation in 96-well plates

To explore the inhibitory effects of *Lactobacillus* on EHEC biofilm formation, we isolated the cell extracts cb-EPS and r-EPS from *L. acidophilus* A4. Biofilm formation was clearly inhibited in 1/2-diluted LB medium when all components were added to a 48 h biofilm at a concentration of 0.1–10.0 mg/ml (Fig. 1A and B). All active components examined potently inhibited biofilm formation in a dose-dependent manner. Interestingly, in polystyrene wells that contained 1.0 mg/ml r-EPS, EHEC biofilm formation decreased by 87%, which was a more significantly decrease when compared to

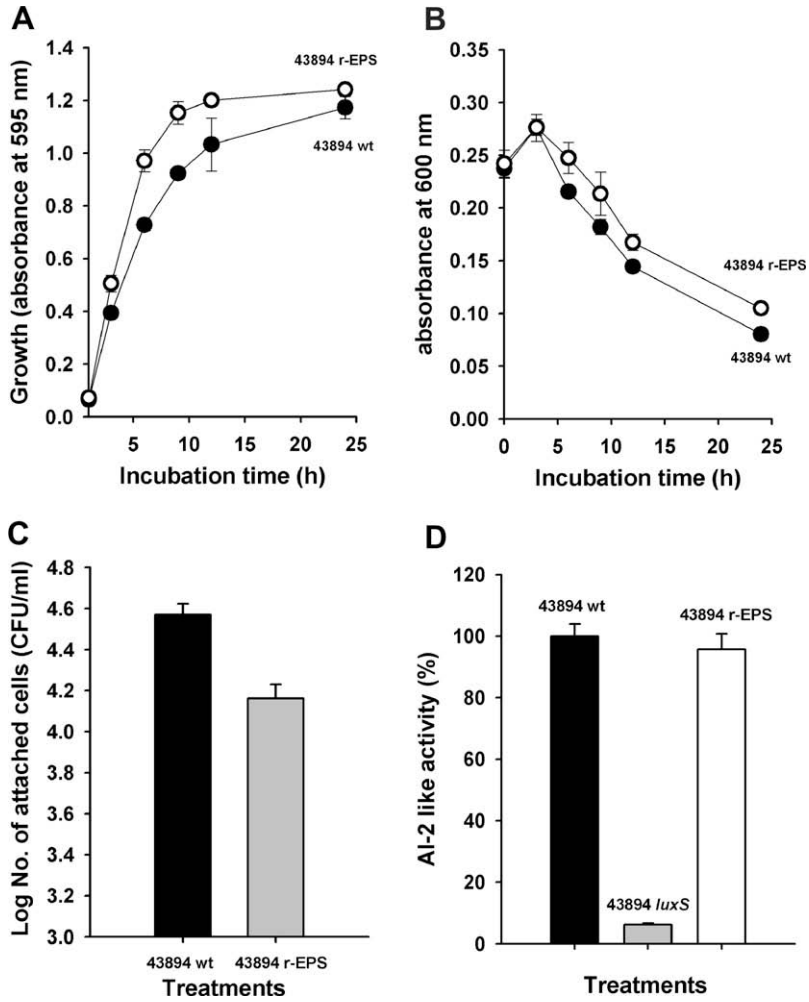

Fig. 2. Growth curve (A), autoaggregation (B), initial attachment (C), and AI-2 like activity (D) of EHEC 43894 in the presence or absence of 1.0 mg/ml r-EPS. Means and standard deviations of three independent experiments are shown.

the decrease induced by the other components at the same concentration. Similarly, EHEC biofilm formation was also inhibited by 94% on PVC surfaces under the same conditions. These results suggest that r-EPS is potentially useful in preventing biofilm formation on a plastic surface.

We then examined the effect of these components on the growth of EHEC O157:H7 43894 in LB medium or 1/2-diluted LB medium at 30 or 37 °C. None of these active components significantly affected the growth yield of EHEC in both LB and 1/2-diluted LB medium at 30 and 37 °C to the same extent as r-EPS (data not shown except 1/2-diluted LB at 37 °C in Fig. 2A). Therefore, biofilm inhibition did not result from a growth defect caused by the bactericidal activity of r-EPS. On a PVC surface, microscopic images showed that only a small number of EHEC cells had attached after 24 and 48 h, whereas EHEC had formed clear air-surface aggregates (Fig. 1C).

Prior to this study, only the rhamnolipid of *Pseudomonas aeruginosa* PAO1 [16] and the lipo-peptides produced by several Gram-positive bacteria [17] had been shown to inhibit biofilm formation. More recently, Valle et al. [4] also found that the treatment of abiotic surfaces with polysaccharides has a long-lasting effect sufficient to significantly inhibit mature biofilm development by a broad range of bacteria. However, there have been only a few reports on biofilm inhibition by food-grade materials. To the best of our knowledge, this is the first report to investigate biofilm inhibition by active components isolated from *Lactobacillus* strains.

Effects of r-EPS on the bacterial cell surface and cell signal systems

In *E. coli*, a number of different mechanisms have been described that promote the spontaneous formation of multi-cellular clusters [18]. Recent observations lend strong support to the notion that initial attachment and aggregation of pathogenic bacteria are important virulence mechanisms [19,20]. The autoaggreation ability of EHEC was reduced to 75% of the control level when 1.0 mg/ml of r-EPS was added (Fig. 2B). In addition, attachment experiments were performed using human epithelial HT-29 cells as an intestinal model to determine whether r-EPS can reduce the attachment ability of EHEC O157:H7, an important prerequisite for biofilm formation. The r-EPS (1.0 mg/ml) slightly decreased the initial attachment of EHEC O157:H7 after 3 h of incubation (Fig. 2C). Several mutations in genes critical for attachment and microcolony formation have been shown to reduce early attachment to dramatically low (<10%) levels compared with those of the wild type, and a large number of genes identified as important for biofilm formation have been shown to be involved in one of

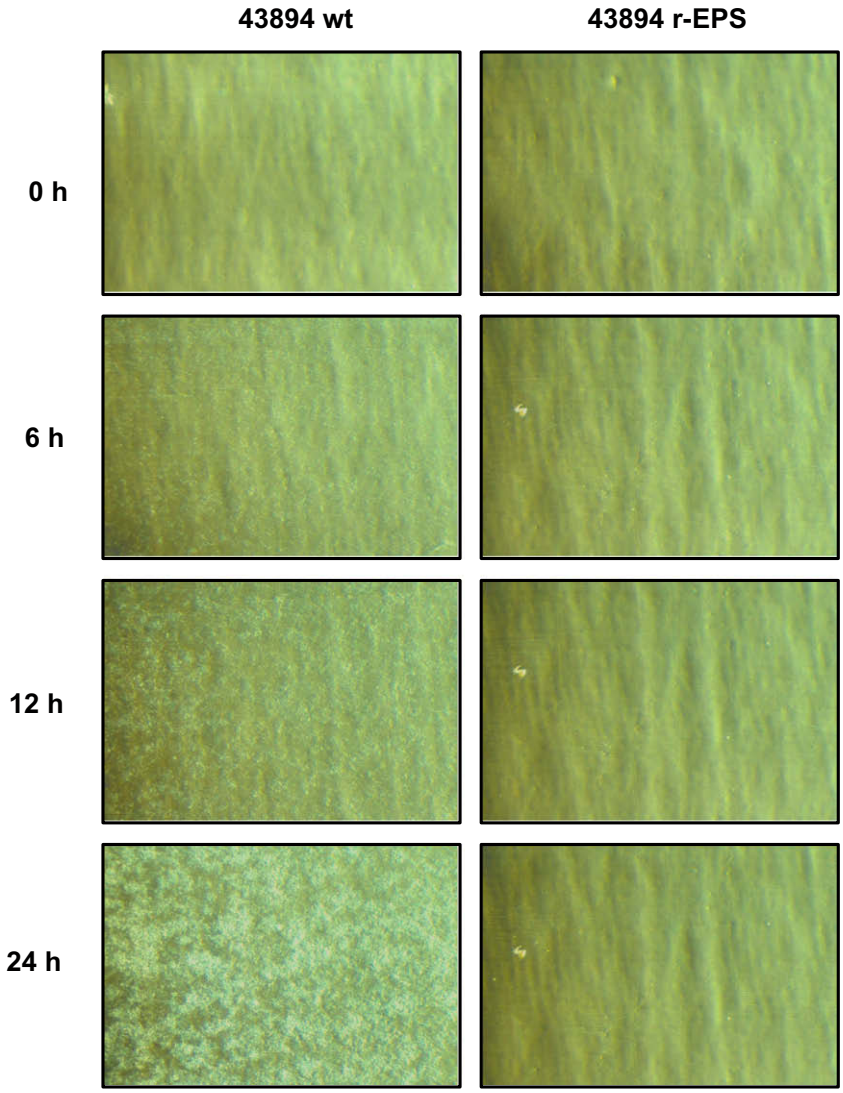

Fig. 3. Quantification of biofilm formation using glass capillary tubes and a continuous-flow system. The biofilms developed by wild-type EHEC 43894 in the presence or absence of 1.0 mg/ml r-EPS were observed at different times as indicated.

these two forms of motility [21,22]. Therefore, our results demonstrate, at least in part, that r-EPS isolated from *L. acidophilus* A4 may inhibit the initial attachment and autoaggregation of EHEC O157:H7 during biofilm development by partially affecting bacterial surface properties.

Previously, Ren and colleagues showed that furanone and ursolic acid inhibit genes related to AI-2 quorum sensing; hence, it appears that both furanone and ursolic acid inhibit biofilm formation [6,7]. To investigate whether r-EPS affects the AI-2 quorum sensing system, cell-free supernatants from wild-type EHEC 43894 containing AI-2-like activity were added to *V. harveyi* BB170 cultures, and inhibition by r-EPS (0, 0.1, 1.0, and 10.0 mg/ml) was examined. The *luxS* insertinal mutant was used as a negative control [23]. Consistent with the observation that ursolic acid has anti-biofilm activity, as described by Ren et al. [7], we did not observed any profound changes in the AI-2 like activity compared with that of the wild type in the presence of r-EPS (Fig. 2D). Therefore, the reduction in biofilm formation by r-EPS is not caused by the repression of the AI-2-like activity.

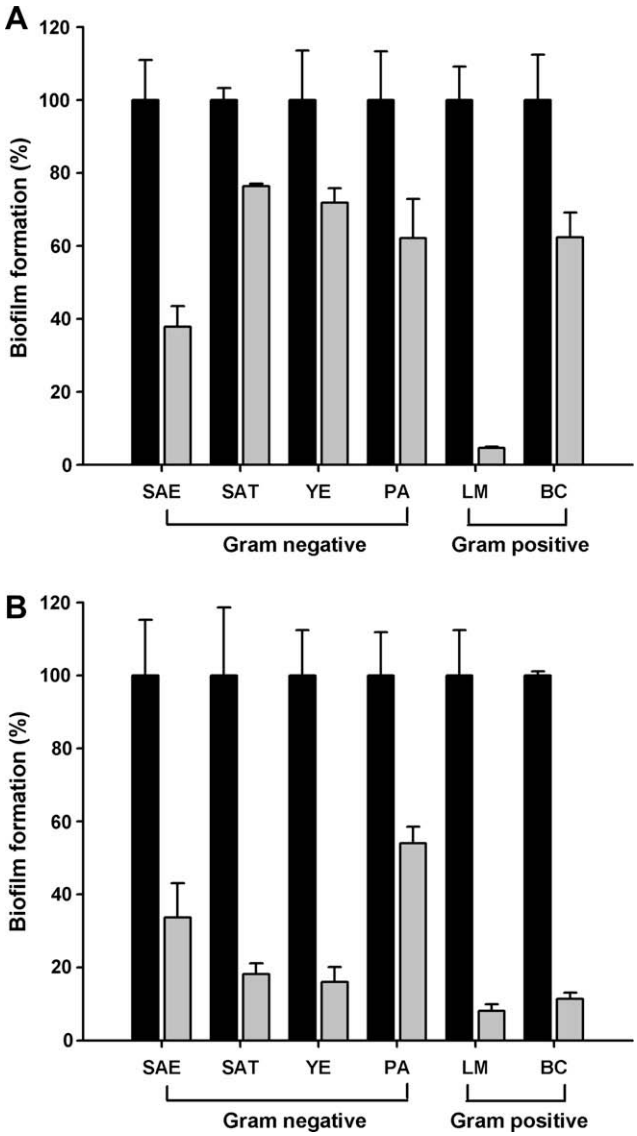

Fig. 4. Effect of r-EPS on biofilm formation of a number of different bacteria. Biofilm formation in the absence (black bars) or presence (gray bars) of 1.0 mg/ml r-EPS on (A) polystyrene and (B) PVC surfaces. Means and standard deviation (SD) of three independent experiments are shown.

Effects of r-EPS on biofilm formation under flow chamber

To further examine the inhibitory effect of r-EPS on the EHEC biofilm architecture, as well as to corroborate the 96-well plate CV staining results, continuous-flow systems in capillary tubes was used to establish biofilms of EHEC O157:H7 43894. In glass capillary tubes, cells attached to the glass surface and began to make cell clusters within 12 h in a similar manner to the controls in the absence of r-EPS. These cell clusters matured to larger organized microcolonies within 24 h and formed a very thick biofilm. In contrast, when 1.0 mg/ml r-EPS was added, EHEC biofilm formation in the presence of r-EPS was observed with dramatically decreased biomass compared to that of the controls (Fig. 3). Taken together, these results suggest that r-EPS isolated from *L. acidophilus* A4 may strongly exert anti-biofilm activity by weakening cell surface modifications or by reducing cell to cell surface interactions.

Specific genes regulation in the presence of r-EPS

To determine the genes in EHEC O157:H7 that were controlled by r-EPS, we performed a whole-transcriptome study using DNA microarray. After exposure of 1.0 mg/ml r-EPS to EHEC O157:H7, a total of 25 genes were found to be repressed by over 2.0-fold while 11 genes were induced by over 2.0-fold (Supplementary Table 1). Among these genes, the curli production genes, including *crl*, (2.0-fold), *csgA* (1.8-fold), and *csgB* (1.7-fold,) and chemotaxis gene, including *cheY* (2.2-fold), were significantly repressed in the presence of r-EPS. Curli genes are mainly composed of a major (CsgA) and minor (CsgB) subunit and influence the attachment and biofilm properties of *E. coli* strains, including EHEC O157:H7 [24]. Also, Crl, which interacts with RpoS to facilitate RpoS binding to the *csgAB* promoter site, is required in most strains for curli expression [25]. In addition, the *cheY* gene encodes a response regulator of the Che chemotaxis system in bacteria [26]. Recently, it was shown that a *P. aeruginosa cheY* mutant was unable to form normal mushroom-shaped structures in mono-strain biofilms [27]. Therefore, we found that r-EPS may directly control biofilm formation through regulation of curli production and chemotaxis as biofilm-associated virulence factors in EHEC O157:H7.

Anti-biofilm activity of r-EPS in Gram-positive and -negative bacteria

To assess the inhibition spectrum of r-EPS on biofilm formation, we investigated the effect of r-EPS (1.0 mg/ml) on biofilm formation of several pathogenic bacteria (*Salmonella enteritidis*, *Salmonella typhimurium* KCCM 11806, *Yersinia enterocolitica, P. aeruginosa* KCCM 11321, *Listeria monocytogenes* ScottA, and *Bacillus cereus*). From these experiments, r-EPS was shown to significantly decrease biofilm formation in all bacteria tested on both polystyrene and PVC surfaces (Fig. 4). These results indicated that r-EPS has broad activity against biofilm formation in both Gram-negative and -positive bacteria.

In conclusion, novel antagonists with the potential to remove mature biofilms are needed, but, until now, only a few laboratories have focused on using natural compounds to inhibit biofilm formation. Therefore, r-EPS isolated from *L. acidophilus* in this study may be applied to various food industries, and used as food-grade adjuncts for microbial biofilm control.

Acknowledgments

This work was supported by a Grant (20050401-034-698-151-00-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea. Y. Kim was supported by the Korea Re-

search Foundation Grant funded by the Korean Government (MOEHRD) (KRF-F00025).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.12.053.

References

[1] F. Trémoulet, O. Duché, A. Namane, B. Martinie, J.C. Labadie, A proteomic study of *Escherichia coli* O157:H7 NCTC 12900 cultivated in biofilm or in planktonic growth mode, FEMS Microbiol. Lett. 215 (2002) 7–14.
[2] Y.H. Kim, Y. Lee, S. Kim, J. Yeom, S. Yeom, K.B. S, S. Oh, S. Park, C.O. Jeon, W. Park, The role of periplasmic antioxidant enzymes (superoxide dismutase and thiol peroxidase) of the Shiga toxin-producing *Escherichia coli* O157:H7 in the formation of biofilms, Proteomics 6 (2006) 6181–6193.
[3] L. Hall-Stoodley, J.W. Costerton, P. Stoodley, Bacterial biofilms: from the natural environment to infectious diseases, Nat. Rev. Microbiol. 2 (2004) 95–108.
[4] J. Valle, S. Da Re, N. Henry, T. Fontaine, D. Balestrino, P. Latour-Lambert, J.M. Ghigo, Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide, Proc. Natl. Acad. Sci. USA 103 (2006) 12558–12563.
[5] M. Labbate, S.Y. Queck, K.S. Koh, S.A. Rice, M. Givskov, S. Kjelleberg, Quorum sensing-controlled biofilm development in *Serratia liquefaciens* MG1, J. Bacteriol. 186 (2004) 692–698.
[6] D. Ren, L.A. Bedzyk, R.W. Ye, S.M. Thomas, T.K. Wood, Differential gene expression shows natural brominated furanones interfere with the autoinducer-2 bacterial signaling system of *Escherichia coli*, Biotechnol. Bioeng. 88 (2004) 630–642.
[7] D. Ren, R. Zuo, A.F. González Barrios, L.A. Bedzyk, G.R. Eldridge, M.E. Pasmore, T.K. Wood, Differential gene expression for investigation of *Escherichia coli* biofilm inhibition by plant extract ursolic acid, Appl. Environ. Microbiol. 71 (2005) 4022–4034.
[8] S. Oh, S.H. Kim, R.W. Worobo, Characterization and purification of a bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus* 30SC, J. Dairy Sci. 83 (2000) 2747–2752.
[9] Y. Kim, K.S. Han, J.Y. Imm, S. Oh, S. You, S. Park, S.H. Kim, Inhibitory effects of *Lactobacillus acidophilus* lysates on the cytotoxic activity of shiga-like toxin 2 produced from *Escherichia coli* O157:H7, Lett. Appl. Microbiol. 43 (2006) 502–507.
[10] J.U. Kim, Y. Kim, K.S. Han, S. Oh, K.Y. Whang, J.N. Kim, S.H. Kim, Function of cell-bound and released exopolysaccharides produced by *Lactobacillus rhamnosus* ATCC 9595, J. Microbiol. Biotechnol. 16 (2006) 939–945.
[11] P. Ruas-Madiedo, M. Gueimonde, C.G. de los Reyes-Gavilán, S. Salminen, Short communication: effect of exopolysaccharide isolated from "viili" on the adhesion of probiotics and pathogens to intestinal mucus, J. Dairy Sci. 89 (2006) 2355–2358.
[12] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colometric method for determination of sugar and related substances, Anal. Chem. 28 (1956) 350–356.
[13] T. Toba, T. Kotani, S. Adachi, Capsular polysaccharide of a slime-forming *Lactococcus lactis* ssp. *cremoris* LAPT 3001 isolated from Swedish fermented milk 'langfil', Int. J. Food Microbiol. 12 (1991) 167–172.
[14] C. Beloin, K. Michaelis, K. Lindner, P. Landini, J. Hacker, J.M. Ghigo, U. Dobrindt, The transcriptional antiterminator RfaH represses biofilm formation in *Escherichia coli*, J. Bacteriol. 188 (2006) 1316–1331.
[15] Y. Kim, S. Oh, E.Y. Ahn, J.Y. Imm, S. Oh, S. Park, S.H. Kim, Proteome analysis of virulence factor regulated by autoinducer-2 like activity in *Escherichia coli* O157:H7, J. Food Prot. 20 (2007) 300–307.
[16] M.E. Davey, N.C. Caiazza, G.A. O'Toole, Rhamnolipid surfactant production affects biofilm structure in *Pseudomonas aeruginosa* PAO1, J. Bacteriol. 185 (2003) 1027–1036.
[17] J.R.I. Mireles, A. Toguchi, R.W. Harshey, *Salmonella enterica* Serovar Typhimurium swarming mutants with altered biofilm-forming abilities: surfactin inhibits biofilm formation, J. Bacteriol. 183 (2001) 5848–5854.
[18] M.A. Schembri, G. Christiansen, P. Klemm, FimH-mediated autoaggregation of *Escherichia coli*, Mol. Microbiol. 41 (2001) 1419–1430.
[19] M.A. Schembri, L. Hjerrild, M. Gjermansen, P. Klemm, Differential expression of the *Escherichia coli* autoaggregation factor antigen 43, J. Bacteriol. 185 (2003) 2236–2242.
[20] O. Sherlock, R.M. Vejborg, P. Klemm, The TibA adhesin/invasin from enterotoxigenic *Escherichia coli* is self recognizing and induces bacterial aggregation and biofilm formation, Infect. Immun. 73 (2005) 1954–1963.
[21] G.A. O'Toole, R. Kolter, Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development, Mol. Microbiol. 30 (1998) 295–304.
[22] M.M. Ramsey, M. Whiteley, *Pseudomonas aeruginosa* attachment and biofilm development in dynamic environments, Mol. Microbiol. 53 (2004) 1075–1087.
[23] Y. Kim, S. Oh, S. Park, J.B. Seo, S.H. Kim, *Lactobacillus acidophilus* reduces expression of enterohemorrhagic *Escherichia coli* O157:H7 virulence factors by inhibiting autoinducer-2-like activity, Food Control. 19 (2008) 1042–1050.
[24] R. Van Houdt, C.W. Michiels, Role of bacterial cell surface structures in *Escherichia coli* biofilm formation, Res. Microbiol. 156 (2005) 626–633.
[25] A. Arnqvist, A. Olsén, J. Pfeifer, D.G. Russell, S. Normark, The Crl protein activates cryptic genes for curli formation and fibronectin binding in *Escherichia coli* HB101, Mol. Microbiol. 6 (1992) 2443–2452.
[26] J. Stock, M. Surette, Bacterial chemotaxis. The motor connection, Curr. Biol. 4 (1994) 143–144.
[27] K.B. Barken, S.J. Pamp, L. Yang, M. Gjermansen, J.J. Bertrand, M. Klausen, M. Givskov, C.B. Whitchurch, J.N. Engel, T. Tolker-Nielsen, Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in *Pseudomonas aeruginosa* biofilms, Environ. Microbiol. 10 (2008) 2331–2343.