

Inhibition of *Escherichia coli* O157:H7 Attachment by Interactions Between Lactic Acid Bacteria and Intestinal Epithelial Cells

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The intestinal epithelial cell (IEC) layer of the intestinal tract makes direct contact with a number of microbiota communities, including bacteria known to have deleterious health effects. IECs possess innate protective strategies against pathogenic challenge, which primarily involve the formation of a physicochemical barrier. Intestinal tract mucins are principal components of the mucus layer on epithelial surfaces, and perform a protective function against microbial damage. However, little is currently known regarding the interactions between probiotics/pathogens and epithelial cell mucins. The principal objective of this study was to determine the effects of *Lactobacillus* on the upregulation of MUC2 mucin and the subsequent inhibition of *E. coli* O157:H7 attachment to epithelial cells. In the current study, the attachment of *E. coli* O157:H7 to HT-29 intestinal epithelial cells was inhibited significantly by *L. acidophilus* A4 and its cell extracts. It is also important to note that the expression of MUC2 mucin was increased as the result of the addition of *L. acidophilus* A4 cell extracts (10.0 mg/ml), which also induced a significant reduction in the degree to which *E. coli* O157:H7 attached to epithelial cells. In addition, the mRNA levels of IL-8, IL-1 β , and TNF- α in HT-29 cells were significantly induced by treatment with *L. acidophilus* A4 extracts. These results indicate that MUC2 mucin and cytokines are important regulatory factors in the immune systems of the gut, and that selected lactobacilli may be able to induce the upregulation of MUC2 mucin and specific cytokines, thereby inhibiting the attachment of *E. coli* O157:H7.

Keywords: *E. coli* O157:H7, *Lactobacillus acidophilus*, attachment, MUC2, cytokine, reverse-transcription polymerase chain reaction

Probiotic bacteria, including *Lactobacillus acidophilus*, are live microorganisms that occur as natural gut flora and evidence no pathogenicity [23, 24]. Rather, these bacteria are crucial to the health and well-being of the host [21]. It has been definitively established that these bacteria represent effective tools for controlling pathogen overgrowth, as well as the control or prevention of infections [17, 21, 34]. Indeed, numerous *in vitro* and *in vivo* studies conducted using different probiotic bacteria genera have demonstrated the ability of these bacteria to interfere with both the growth and virulence of a variety of pathogens [7, 26, 33].

The intestinal epithelial cell (IEC) layer of the intestinal tract is strategically located between the many microbes and antigens of the intestinal lumen, and the inflammatory and immune effectors of host cells [27]. IECs actively generate signals for communication with mucosal immune and inflammatory cells [36]. Epithelial microbial cross-talk occurs constantly, and is presumably mediated, at least in part, by Toll-like receptors (TLR) [29], putative internal NOD receptors [14], and other mechanisms. Interactions between enteropathogenic bacteria and the host mucosa have been extensively documented. Pathogens utilize highly specialized adaptive strategies, thereby permitting them to colonize the mucosa of the gut [1]. Cytokines and chemokines expressed by epithelial cells in response to attachment attract and activate neutrophils and monocytes within the adjacent intestinal mucosa. Thus, the processing and signaling pathways exploited by epithelial cells constitute primary components of the innate and adaptive defense mechanisms operant in the earliest phases after pathogenic attachment [16].

However, among other factors, the IEC-derived mucus is the principal interface between the immune system and the luminal environments, including dynamic microbiotic ecosystems [27]. Mucins are high-molecular-weight glycoproteins, which are synthesized and secreted by the

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IECs of a number of organ systems, including the intestinal tract. The mucins are characterized by their large size, high carbohydrate content, and *O*-glycosidic bonds [27]. Among the human mucin genes, MUC2 and MUC3 are the predominant ileocolonic mucins. In particular, the MUC2 mucin polypeptide harbors a repetitive peptide comprised of 23 amino acids, which is rich in threonine and proline residues, is heavily glycosylated, and is flanked by cysteine-rich domains [10]. The MUC2 gene is expressed in the goblet cells of the small and large intestines, and MUC2 mucins may be the primary secreted mucin component in the colon [13]. Interestingly, the results of a previous study showed that probiotics such as *Lactobacillus rhamnosus* GG were capable of binding to specific receptor sites on the IECs, and stimulated the upregulation of MUC2 [28]. In addition, Mack *et al.* [27] also determined that the ability of the *L. plantarum* 299v strain to inhibit the attachment of pathogenic *E. coli*, including the O157:H7 serotype, to intestinal epithelial cells is attributable to the ability of that strain to augment the expression of the intestinal mucins, MUC2 and MUC3.

The principal objective of this study was to determine the effects of lactobacilli and their active components on the modulation of MUC2 and cytokine response, and the subsequent inhibition of *E. coli* O157:H7 attachment to epithelial cells.

MATERIALS AND METHODS

Bacteria and Cell Culture

The bacterial strains employed in this study (Table 1) were obtained from the Dairy Food Microbiology Laboratory at Korea University (Seoul, Korea). The *E. coli* O157:H7 strains were routinely cultured at 37°C in Luria-Bertani broth (Difco, Sparks, MD, U.S.A.) with agitation (175 rpm). *Lactobacillus* strains were cultured in Man, Rogosa, Sharpe broth (MRS; Difco, U.S.A.) for 18 h at 37°C. The HT-29 cell lines were acquired from the Korea Culture Type Collection (KCTC, Korea). The cells were routinely cultured in RPMI 1640 medium (Gibco BRL, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), penicillin G (100 IU/ml), and streptomycin (100 mg/ml) at 37°C in an atmosphere of 5% CO₂.

Additionally, HT-29 DM2, which are HT-29 cells in which the MUC2 gene is suppressed, were constructed *via* an RNA interference technique using the mammalian expression vector pSUPER.retro.puro (OligoEngine, U.S.A.; Kim and Kim, unpublished data). For stable expression, HT-29 DM2 cells were cultured in the presence of puromycin (1 mg/ml).

Preparation of Cell Extracts from Lactobacilli

The cell extracts, polysaccharides, and lipids were isolated *via* the methods described previously by Choi *et al.* [3]. Finally, each of the extracts was resuspended with RPMI 1640 medium at a concentration of 10 mg/ml (dried sample weight), adjusted to pH 7.2, sterilized *via* filtration (0.22 µm pore size; Sartorius, Gottingen, Germany), and stored at -70°C until used in further experiments.

Attachment Assays

HT-29 or HT-29 DM2 monolayers were prepared as described above. For the pretreatment experiments (exclusion assay), lactobacilli at a concentration of 10⁹ CFU/ml, or 10.0 mg/ml of cell extract (approximately correspondence to 10⁹ CFU cells) in an antibiotic-free medium, were added for 2 h at 37°C in an atmosphere of 5% CO₂. After incubation, the monolayers of HT-29 cells were washed three times in PBS, and 10⁶ CFU/ml of *E. coli* O157:H7 cells in the identical medium were added to each well, and then incubated for an additional 3 h. For the cotreatment experiments (competition assay), lactobacilli and *E. coli* O157:H7 strain cells (10⁹ CFU/ml and 10⁶ CFU/ml in antibiotics-free medium, respectively) were mixed and incubated for 3 h. The monolayers were then washed five times in PBS in order to remove any nonattached bacteria. The adherent cells were released from well plates using 0.1 ml of trypsin-EDTA and incubated for 5 min at 37°C. Serial dilutions of mixtures were plated on MacConkey agar and incubated overnight at 37°C in an anaerobic culture system (BBL Gas-Pak System, Sparks; MD, U.S.A.) for subsequent CFU quantification.

In analogous experiments, fluorescent images of *E. coli* O157:H7 43894-GFP [22] attached to the HT-29 cells were assessed directly *via* fluorescence microscopy (Olympus IX71, Tokyo, Japan) at identical incubation times as were used to observe changes in the attachment phenotypes.

Semiquantitative RT-PCR Analysis

The regulation of cytokine and motility-associated genes were investigated *via* semiquantitative RT-PCR techniques. Total RNA was isolated from each of the treated samples using an RNeasy Total RNA Kit (Qiagen, Germany) in accordance with the manufacturer's

Table 1. Bacterial strains used in this study.

Strains	Description	References
43889	<i>E. coli</i> O157:H7 (stx-2)	ATCC ^a
43894	<i>E. coli</i> O157:H7 (stx-1 and stx-2)	ATCC
43894-GFP	GFP-expressing 43894	[22]
A4	<i>Lactobacillus acidophilus</i> (from pig)	Lab isolate. [21]
30SC	<i>Lactobacillus acidophilus</i> (from calf)	Lab isolate. [34]
107A	<i>Lactobacillus acidophilus</i> (from pig)	Lab isolate.
606	<i>Lactobacillus acidophilus</i> (from human)	Lab isolate. [3]
GG (ATCC 53103)	<i>Lactobacillus rhamnosus</i>	ATCC

^aATCC, American Type Culture Collection.

Table 2. Oligonucleotides primers used for RT-PCR analysis of MUC2 and various cytokines gene expression.

Genes	Sequence of PCR Primers	Annealing temp.	Amplified fragment	References
MUC2	Forward: 5'-CTGCACCAAGACCGTCCTCATG-3' Reverse: 5'-GCAAGGACTGAACAAAGACTCAGA-3'	54°C	401 bp	[8]
IL-1 β	Forward: 5'-GATCATCTGTCTCTGAATCA-3' Reverse: 5'-TCCAGATTATGTAATGCAGC-3'	58°C	387 bp	[10]
IL-8	Forward: 5'-CTGGCCGTGGCTCTCTTGGCAGCCTTCCTG-3' Reverse: 5'-GGCAACCCTACAACAGACCCACACAATACA-3'	58°C	395 bp	[10]
IL-10	Forward: 5'-TGATGTCTGGGTCTTGGTTC-3' Reverse: 5'-GCCTAACATGCTTCGAGATC-3'	58°C	204 bp	[10]
IL-12	Forward: 5'-CGTAGAATTGGATTGGTATCCGG-3' Reverse: 5'-GCTCTTGGCCCTGGACCTGAACGC-3'	58°C	702 bp	[10]
TNF- α	Forward: 5'-CAGAGGGAAGAGTTCCCCAG-3' Reverse: 5'-CCTTGGTCTGGTAGGAGACG-3'	58°C	324 bp	[10]
IFN- γ	Forward: 5'-ATATCTTGGCTTTTCAGCTC-3' Reverse: 5'-CTCCTTTTTTCGCTTCCCTGT-3'	58°C	489 bp	[10]
TLR-2	Forward: 5'-GCCAAAGTCTTGATTGATTGG-3' Reverse: 5'-TTGAAGTCTCCAGCTCCTG-3'	54°C	347 bp	[2]
TLR-4	Forward: 5'-TGGATACGTTTCCTTATAAG-3' Reverse: 5'-GAAATGGAGGCACCCCTTC-3'	54°C	548 bp	[2]
β -Actin	Forward: 5'-CAAGGCCAACCGCGAGAAG-3' Reverse: 5'-CAGGGTACATGGTGGTGCC-3'	55°C	586 bp	[4]

recommendations. One μ g of total RNA was reverse-transcribed in 20 μ l of reaction mixtures with an AMV First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). Then, cDNA was amplified using *Taq* DNA polymerase (TaKaRa, Tokyo, Japan). Four min of denaturation at 94°C was followed by 25 of the following cycles: 45 s of denaturation at 94°C, 45 s of annealing at 54 to 58°C (Table 2), and 1 min of elongation at 72°C. The final cycle was followed by a 7 min final elongation step at 72°C. The oligonucleotide sequences (Bioneer, Daejun, Korea) utilized for the amplification of each of the gene fragments are provided in Table 2. The RT-PCR products were electrophoresed in 1.5% agarose gel and visualized *via* ethidium bromide (EtBr) staining. The intensity of each band was determined *via* densitometric gel analysis using a Kodak DC290 zoom digital camera and Kodak 1D image analysis software (Eastman Kodak Company, Rochester, NY, U.S.A.).

Statistical Analysis

All experiments were conducted at least in triplicate. The effects of each of the treatments were analyzed by ANOVA, followed by Duncan's test, using the SAS software package (Version 9.1; SAS Inc., Cary, NC, U.S.A.). The level of significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Inhibitory Effects of *Lactobacillus* Strains on *E. coli* O157:H7 Attachment

Many researchers have previously demonstrated protective effects against the attachment of a variety of enteric pathogens, including *E. coli* O157:H7, as the consequence of acidification with lactic acid [33], secreted non-acidic products [4], and

interference with attachment to receptors or spaces, all of which may occur both directly and indirectly [12]. The *L. acidophilus* strains in this study were evaluated with regard to their ability to inhibit the attachment of *E. coli* O157:H7 43889 and 43894 to HT-29 cells. *L. rhamnosus* GG was utilized as a positive control [12]. In both the pre- and cotreatment experiments, all tested *L. acidophilus* at a concentration of 10^9 CFU/ml resulted in significant reductions in the attachment of *E. coli* O157:H7 43889 and 43894 to HT-29 cells (Fig. 1). On the other hand, there were no previous results on the other doses such as 10^6 , 10^7 , and 10^8 CFU/ml populations and the inhibition of attachment of *E. coli* O157:H7 was not observed in post-treatment assays (LAB treatment after *E. coli* O157:H7 2 h attachment; data not shown).

The results of the present data show that, in both the pre- and cotreatment experiments, *L. acidophilus* A4 was discovered to result in the most profound attachment inhibitory effects in the *E. coli* O157:H7 strain. Combined with the data obtained in the present and preliminary experiments (acid and bile tolerance, bacteriocin activity, and cholesterol reduction ability; data not shown), *L. acidophilus* A4 was finally selected for use in further studies.

Importantly, we also observed that the 10.0 mg/ml of cell extracts (pH 6.5) isolated from *L. acidophilus* A4 inhibited the attachment of *E. coli* O157:H7 strains in a time-dependent manner (Figs. 2A and 2B). Additionally, GFP-expressing *E. coli* O157:H7 strains were utilized to visualize alterations in the attaching phenotype. The O157:H7 strain formed attachment aggregates more rapidly, whereas reduced attachment was observed by the presence

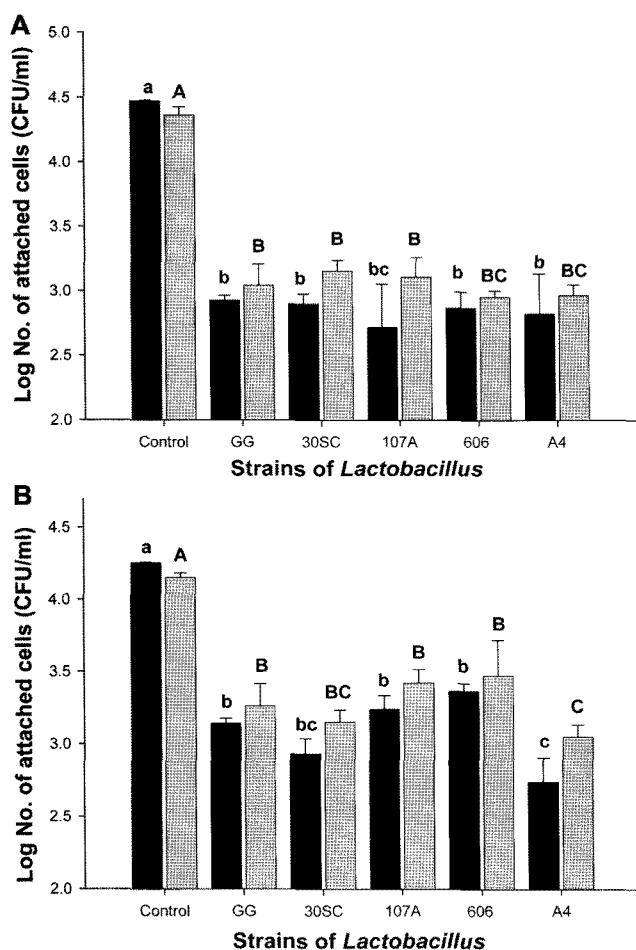


Fig. 1. Effects of *L. acidophilus* strains on the attachment of *E. coli* O157:H7 ATCC 43889 (A) and 43894 (B) to HT-29 cells (Black bars: pretreatments; grey bars: cotreatments).

For the pretreatment experiments, lactobacilli at a concentration of 10^9 CFU/ml in an antibiotic-free medium were added for 2 h at 37°C in an atmosphere of 5% CO₂. After incubation, the monolayers of HT-29 cells were washed three times in PBS, and 10^6 CFU/ml of *E. coli* O157:H7 cells in the identical medium was added to each well, and then incubated for an additional 3 h. In the case of cotreatment experiments, lactobacilli and *E. coli* O157:H7 strain cells (10^9 CFU/ml and 10^6 CFU/ml in antibiotics-free medium, respectively) were mixed and incubated for 3 h. Serial dilutions of mixtures were plated on MacConkey agar and incubated overnight at 37°C in an anaerobic culture system (BBL Gas-Pak System; Sparks, MD, U.S.A.) for subsequent CFU quantification. Error bars indicate the standard deviation (SD) between independent experiments, and different letter superscripts indicate differences at $p < 0.05$.

of the 10.0% (w/v) cell extract, coupled with a significant attenuation of the attachment aggregates (Fig. 2C).

However, the supernatants (adjusted to pH 6.5) of the *L. acidophilus* cultures and the crude polysaccharide or lipid components isolated from the cell extracts of *L. acidophilus* A4 appeared to exert no inhibitory effects on the attachment of either *E. coli* O157:H7 or 43894 (Fig. 3). In addition, profound inhibition of *E. coli* O157:H7 attachment only was observed in the cotreatment experiments, but not in the pretreatment experiments, as was evidenced by the

results of the exclusion assay. Therefore, specific protein-associated components inherent to the cell extracts of *L. acidophilus* may exert some inhibitory effects on the attachment of *E. coli* O157:H7.

Interaction Between MUC2 Expression and *E. coli* O157:H7 Attachment

In the present study, we assessed the regulation of the MUC2 gene in the presence of probiotic bacteria, or their components. By RT-PCR results, the expression of the MUC2 mucin of HT-29 DM2 cells decreased up to 20% compared with that of HT-29 control cells. MUC2 gene expression in the HT-29 cells was evaluated via RT-PCR techniques. MUC2 expression was based on the relative expression as compared with β -actin. As had been expected, the mRNA levels of MUC2 mucin were upregulated (approximately 20–30%) in the presence of cell masses (10^9 CFU/ml) and cell extracts (10.0 mg/ml) of *L. acidophilus* A4 (Fig. 4). These results indicated that the secretion of MUC2 in HT-29 cells could be selectively stimulated by cell masses or cell extracts of *L. acidophilus* A4. Interestingly, as is shown in Fig. 5, *E. coli* O157:H7 adhered more to HT-29 DM2 than to the HT-29 controls. The addition of cell masses and cell extracts significantly reduced the attachment of *E. coli* O157:H7.

The process whereby pathogenic bacteria inflict their characteristic attachment on epithelial cells is a multistage process, the first stage of which is characterized by an initial interaction of bacteria with the IECs. In this step, environmental conditions of IECs such as net fluid and electrolyte secretion are important factors for attachment of pathogens [27]. Among these factors, MUC2 mucins are the main secreted mucus components of the digestive tract from the stomach to the colon, and it consists of a sort of protective elastic and viscous gel consisting of glycoproteins [5, 27]. Several works indicated that the obvious functions of mucin, including MUC2 mucins, are to provide specific protection barriers against bacterial attachment and penetration [28]. However, until the present, there have been only a few reports filed regarding the regulation of mucin expression by probiotic bacteria. Our results also showed that *L. acidophilus* may, at least in part, be capable of inducing the upregulation of MUC2 mucin, thereby inhibiting the attachment capacities of *E. coli* O157:H7.

In agreement with our findings, Mack *et al.* [27] showed that two strains of *Lactobacillus* (*L. plantarum* 299v and *L. rhamnosus* GG) inhibited pathogenic *E. coli*, including *E. coli* O157:H7, via an induction of MUC2 gene expression, using HT-29 IECs [27]. Other studies have indicated that the mRNA expression of the MUC2 gene was stimulated by the presence of probiotic bacteria, as compared with the monolayers of untreated Caco-2 cells [28]. These results indicate that the expression of MUC2 was stimulated by

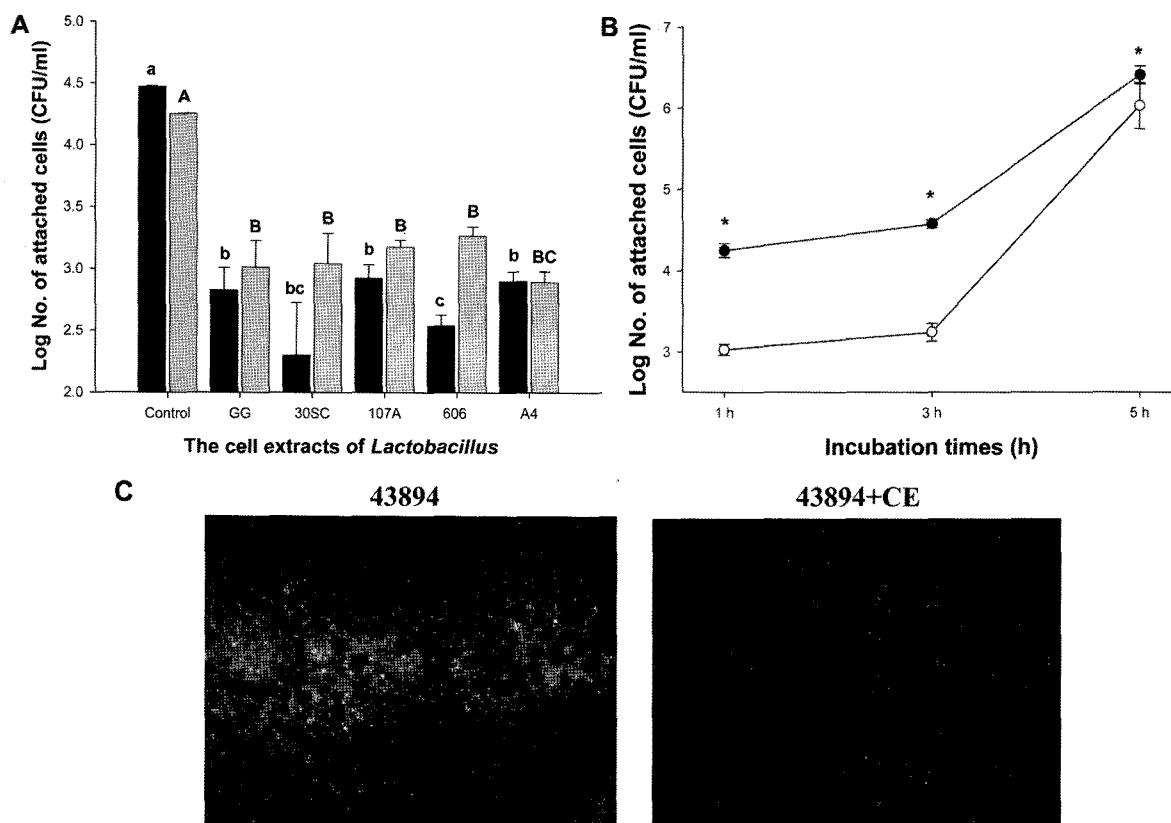


Fig. 2. Effects of the cell extracts (CE) from *L. acidophilus* strains (10.0 mg/ml) on the attachment of *E. coli* O157:H7 43889 (black bars) and 43894 (grey bars) during 3 h cotreatment (A) and the time profiles of the inhibitory effects of CE isolated from *L. acidophilus* A4 (filled symbol: control; open symbols: 10.0 mg/ml CE) during 5 h of co-incubation on *E. coli* O157:H7 43894 attachment (B). Error bars indicate the standard deviation (SD). Different letter superscripts and asterisks indicate differences at $p < 0.05$. C. Fluorescence images of *E. coli* O157:H7 43894 in the absence (left panel) or presence (right panel) of cell extract (10.0 mg/ml) in HT-29 cells at 3 h of cotreatment.

intact cells or its specific components in the cell extracts of *L. acidophilus*, and the regulation of MUC2 mucin by *L. acidophilus* is responsible for the inhibition of the attachment of enteric pathogens, including *E. coli* O157:H7. Our studies are currently under way to evaluate the purification and characterization of MUC2-regulation components in the cell extracts of *L. acidophilus* A4, specifically with regard to protective strategies for inhibiting the attachment of *E. coli* O157:H7.

Cytokine mRNA Expression Mediated by Cell Extracts in HT-29 Cells

The mechanisms by which probiotic bacteria influence the immune system currently remain unknown, but many of them are attributed to increases in innate or acquired immune responses [8]. Recently, it was reported that some live probiotic bacteria including *L. acidophilus*, *L. casei*, and *Bifidobacterium* significantly induced immune response of host in *Caenorhabditis elegans* *in vivo* trials (Ikeda *et al.*, unpublished data). Some researchers have previously reported that Gram-positive bacterial cell wall components,

including those of *Lactobacillus* strains, significantly upregulated the secretion of a variety of proinflammatory cytokines, including TNF- α , IL-1 β , IL-6, and IL-12, in both *in vitro* and *in vivo* trials [11, 20, 35]. Consistent with these reports, in our experiments, the mRNA levels of IL-8, IL-1 β , and TNF- α were induced to a significant extent in the presence of 10.0 mg/ml of *L. acidophilus* A4 cell extracts on the HT-29 cells (Fig. 6). In the case of IL-8, a cytokine belonging to the CXC chemokine family evidences leukocyte chemotactic and stimulatory properties. Recently, the cell extracts isolated from nonpathogenic *E. coli* Nissle 1917 and *Streptococcus faecium* have been shown to exert an effect comparable to that of a known anti-inflammatory drug, inducing IL-8 production in a coculture epithelial cell model [25]. In addition, TNF- α and IL-1 β mRNA levels were induced in the presence of 10.0 mg/ml of *L. acidophilus* A4 cell extracts.

Recently, the ability of probiotic bacteria to modulate immune responses has been demonstrated in a number of studies. Among these, especially, immune-modulation effects of LAB have often been ascribed to a shift of the T

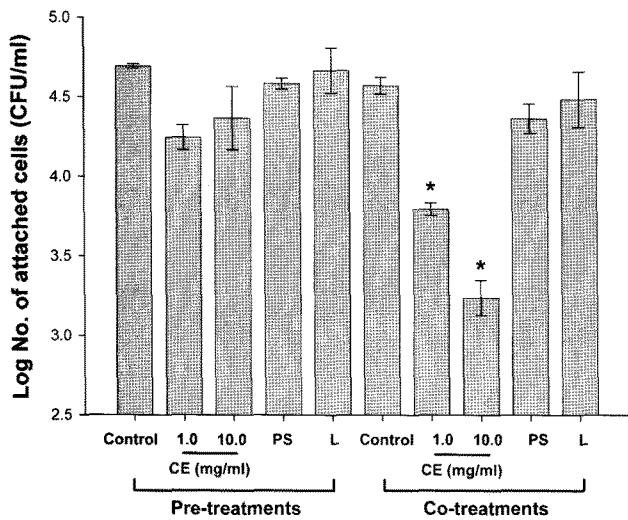


Fig. 3. Inhibitory effects of cell extracts (CE) of *Lactobacillus acidophilus* A4 on the attachment of *E. coli* O157:H7 ATCC 43894 during pre- and cotreatments (PS: polysaccharide components from CE; L: lipid components isolated from CE). Error bars indicate the standard deviation (SD) between independent experiments and the asterisks within same incubation times indicate differences at $p < 0.05$.

helper (Th)1/Th2 balance towards Th1-mediated immunity. Release of the Th1-associated cytokines such as IL-1 β and TNF- α by various cell types was repeatedly demonstrated to be induced by LAB [5, 31]. Consistent with the previous data, the specific inductions of IL-1 β and TNF- α as Th1 immune response were observed, whereas that of IL-10 and IL-12, Th2 immune cytokines, were not determined in the exposure of cell extracts. Interestingly, these cytokines have also been shown to function as activators of MUC2 mRNA expression in intestinal epithelial HT-29 cells and airway NCI-H292 epithelial cells [15, 19].

Toll-like receptors (TLRs) are a family of transmembrane receptors homologous to the *Drosophila* Toll protein. Relatively little remains known regarding the role of TLR-2 signaling in the intestinal epithelium. It has been previously reported that cell wall components from Gram-positive organisms have been identified as ligands for TLR2 [30, 32]. In the present study, we also determined that TLR-2 gene expression in HT-29 cells was upregulated upon exposure to 10.0 mg/ml of *L. acidophilus* A4 cell extracts. Collectively, the results obtained in the present study appear to indicate that the gut immune systems activated by the cell extracts of *L. acidophilus* A4 were, at least in part, activated *via* specific inflammation-associated cytokines and the TLR-2 pathway following the induction of MUC2 mucins, consequently. Future studies should address the regulation of Th1-associated cytokines and TLR-2 in the intestinal epithelium with the association of MUC2 expressions and the immune upregulation of *L. acidophilus* A4, using *in vivo* models such as mouse and

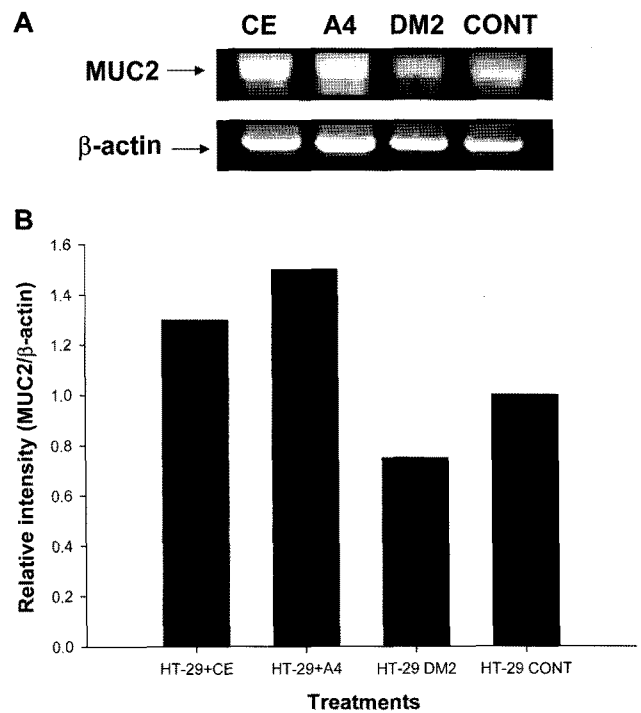


Fig. 4. Effects of *Lactobacillus acidophilus* A4 on MUC2 gene expression.

HT-29 monolayers cultured on 6-well plates were treated for 3 h with cell masses (10^9 CFU/ml) or cell extracts (10.0 mg/ml) as described in the Materials and Methods section. Semiquantitative RT-PCR images (A) and relative intensity (B) analysis for MUC2 were conducted. Data are expressed as MUC2 RT-PCR products normalized to β -actin products.

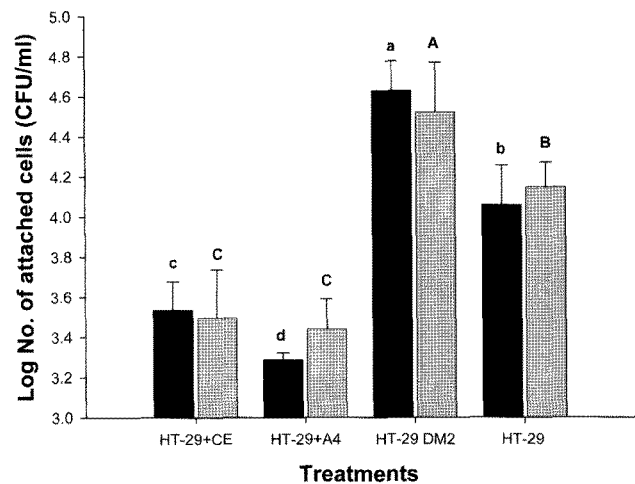


Fig. 5. Effects of MUC2 downregulation on the attachment ability of *E. coli* O157:H7.

Via cotreatment experiments, HT-29 and HT-29 DM2 monolayers cultured on 6-well plates were treated for 3 h with STEC 43889 (black bars) and 43894 (grey bars) at a concentration of 10^6 CFU/ml, as described in the Materials and Methods section. Attachment inhibition tests were conducted *via* the same procedures coupled with cell masses (10^9 CFU/ml) or cell extracts (10.0 mg/ml). Error bars indicate the standard deviation (SD) between independent experiments. Different letter and superscripts indicate differences at $p < 0.05$.

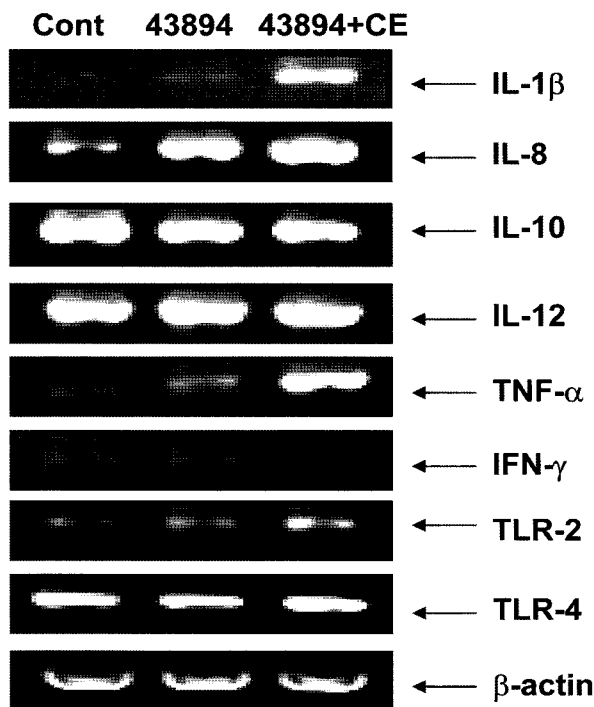


Fig. 6. Cytokine mRNA responses in *E. coli* O157:H7-infected HT-29 cells in the presence or absence of 10.0 mg/ml cell extracts (CE) isolated from *Lactobacillus acidophilus* A4. After 3 h post-attachment, HT-29 cells were excised and the total RNA was extracted. The cDNA constructed using reverse transcriptase (RT) was subjected directly to PCR using the cytokine-specific primers listed in Table 2.

C. elegans, in order to more effectively understand and prevent *E. coli* O157:H7 pathogenesis.

In conclusion, the inhibitory effects of *L. acidophilus* A4 and their cell extracts on the *E. coli* O157:H7 attachment were caused, at least in part, by the upregulation of the MUC2 gene and specific cytokine expression, either directly or indirectly. Until now, the molecular basis of the effects evoked by probiotic bacteria had not been well characterized. Lactic acid bacteria, including *L. acidophilus*, are known to exert a wide range of effects on the immune system, and are designated as GRAS (Generally Recognized as Safe). Further understanding of the molecular foundation of the relationship between probiotic bacteria and the host should help to clarify the contribution of these microorganisms to host physiology, and should also allow for the enhanced application of these approaches in helping with the prevention of *E. coli* O157:H7-associated diseases.

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