Genetics and Molecular Research

Lactic acid bacteria protect human intestinal epithelial cells from *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections

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ABSTRACT. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are opportunistic pathogens that cause nosocomial and food-borne infections. They promote intestinal diseases. Gastrointestinal colonization by *S. aureus* and *P. aeruginosa* has rarely been researched. These organisms spread to extra gastrointestinal niches, resulting in increasingly progressive infections. Lactic acid bacteria are Gram-positive bacteria that produce lactic acid as the major end-product of carbohydrate fermentation. These bacteria inhibit pathogen colonization and modulate the host immune response. This study aimed to investigate the effects of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* on enteric infections caused by the paradigmatic human pathogens *S. aureus* ATCC25923 and *P. aeruginosa* ATCC27853. The effect of whole cells and neutralized cell-free supernatant (CFS) of the lactobacilli on LoVo human carcinoma enterocyte (ATCC CCL-229) infection was analyzed by co-exposure, pre-exposure, and post-exposure studies. Simultaneous application of whole cells and CFS of the lactobacilli significantly eradicated enterocyte infection ($P < 0.05$); however, this effect was not seen when the whole cells and CFS

were added after or prior to the infection ($P > 0.05$). This result could be attributed to interference by extracellular polymeric substances and cell surface hydrophobicity, which resulted in the development of a pathogen that did not form colonies. Furthermore, results of the plate count and LIVE/DEAD *Bac*Light bacterial viability staining attributed this inhibition to a non-bacteriocin-like substance, which acted independently of organic acid and H_2O_2 production. Based on these results, the cell-free supernatant derived from lactobacilli was concluded to restrain the development of *S. aureus* and *P. aeruginosa* enteric infections.

Keywords: Infection; Intestinal epithelial cells; Lactic acid bacteria; *Pseudomonas aeruginosa*; *Staphylococcus aureus*

INTRODUCTION

Antimicrobial resistance is a growing threat to human health; the increase in antimicrobial resistance is a direct consequence of the excessive use of antibiotics. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are well-known opportunistic, potent, biofilm-producing, nosocomial and food-borne pathogens. They generally have the ability to promote intestinal diseases. The use of antibiotics for the treatment of *P. aeruginosa* and *S. aureus* infections in humans remains controversial; however, the increase in antibiotic resistance in these bacterial species is a growing cause for concern.

Human colonization by *S. aureus* is widespread; thirty percent of the population carries *S. aureus* as part of the nasopharyngeal, skin, and intestinal epithelial microbiota (Graham et al., 2006). Research conducted over the past few years has revealed that *S. aureus* colonizes the intestine of several neonates; its presence in the gut during the early developmental stages has also been associated with allergy development (Björkstén et al., 2001). This bacterium causes diseases ranging from skin infections to food-borne illnesses, and severe infections, including endocarditis, osteomyelitis, and sepsis (Lowy, 1998). The biofilm-forming ability of this bacterium depends on the production of polysaccharide intercellular adhesin (PIA), encoded by the *ica*ADBC gene cluster (Cramton et al., 1999).

P. aeruginosa is an aerobic, Gram-negative, ubiquitous bacterium. It has a high degree of adaptability and is resistant to many antibiotics (Pearson et al., 2000). The intestinal carriage of *P. aeruginosa* could be attributed to its opportunistic nature. This bacterium causes enteric disease accompanied by sepsis, which is described as “Shanghai fever”, a syndrome comprising fever, diarrhea, and sepsis; this disease was attributed to *P. aeruginosa* in as early as 1918 (Lepow, 1994). Gastrointestinal colonization with subsequent invasion into the bloodstream is the presumed mechanism underlying *P. aeruginosa* bacteremia in neutropenic patients. Diarrhea caused by *P. aeruginosa* has been observed almost exclusively in patients with prolonged antibiotic exposure (Kim et al., 2001). The development of antibiotic resistance is a serious side effect of current antipseudomonal treatments.

Lactic acid bacteria comprise a group of Gram-positive, non-sporulating rods or cocci, which produce lactic acid as the major end-product of carbohydrate fermentation (Aasen et al., 2000; Suskovic et al., 2001). These bacteria produce a variety of antimicrobial compounds (Bongaerts and Severijnen, 2001; Servin, 2004) such as organic acids, diacetyl compounds, and hydrogen peroxide during lactic fermentation (Lindgren and Dobrogosz, 1990). The production of

organic acids results in a decrease in intestinal pH, and the inhibition of growth of other bacterial pathogens (Fang et al., 1996). In this way, these bacteria impart health and nutritional benefits to the host. They also have the potential to inhibit pathogen colonization and modulate the immune response (Laparra and Sanz, 2009).

Compared to the nasal carriage of *S. aureus* and *P. aeruginosa*, gastrointestinal colonization by *S. aureus* and *P. aeruginosa* has been rarely studied. Herein, we have focused on two paradigmatic human pathogenic bacteria of great medical importance, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These organisms spread from the gastrointestinal tract to extra gastric niches to produce progressive infections. This study aimed to investigate the effects of lactobacilli and their cell-free extracts on *S. aureus* and *P. aeruginosa* (enterocyte) infection. This study was initiated as an attempt to understand and control the continuous increase in antibiotic resistance in these human pathogens.

MATERIAL AND METHODS

Microorganisms and culture conditions

Human bacterial pathogens *(S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853) were obtained from American type culture collection (ATCC), cultured in Luria-Bertani (LB) broth, and incubated at 37°C. *Lactobacillus rhamnosus* CMCC 341 and *Lactobacillus acidophilus* CMCC 878 were acquired from China Medical Culture Collection (CMCC), cultured in de Man Rogosa Sharpe (MRS) broth, and incubated at 37°C.

Preparation of cell-free supernatant (CFS) derived from lactobacilli

The *Lactobacillus* culture, containing 1.5 x 10^8 CFU/mL was incubated overnight. This was then centrifuged at 10,000 *g* for 10 min at 4°C. The resulting supernatant was neutralized, in order to exclude all pH-dependent activities (Ogunbanwo et al., 2003).

Adhesion to an abiotic surface

The preliminary adhesion of human bacterial pathogens to the walls of pre-sterilized 96-well polystyrene microtiter plates was studied using the method described by Maldonado et al. (2007). The plates were subsequently incubated for 24, 48, and 72 h at 37°C. The samples were quantitatively analyzed by adding 33% (v/v) glacial acetic acid (Merck-Millipore, Darmstadt, Germany) per well and measuring OD_{589} by using an enzyme-linked immunosorbent assay (ELISA) reader (STAT-FAX). All assays were performed in triplicate.

Infection of human intestinal cells

LoVo human enterocytes (CCL-229) were obtained from ATCC. The cells were cultured in 24-well plates with Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum and 100 µg/mL penicillin-streptomycin (Invitrogen; Life Technologies, Carlsbad, CA, USA). The cells were incubated until a confluence of 75% was achieved in a 5% CO_2 incubator at 37°C. The cells were washed thrice with phosphate buffered saline (PBS), transferred into DMEM culture medium without serum or antibiotics for 2 h prior to the infection, and subsequently used

in the adhesion experiments. Bacterial pellets (pathogens and lactobacilli) were suspended in serum-antibiotic-free DMEM; the cell concentration was adjusted to 2 x 10^8 CFU/mL prior to the adherence assay. The enterocytes were infected with the pathogens in the presence or absence of lactobacilli in 24-well plates for 24 h. The average number of viable cells/well was determined by the Trypan blue dye exclusion test; this number was used to achieve a multiplicity of infection (MIO) of 1:10. After this incubation period, the non-adherent cells were washed thrice with PBS. The adherent cells were subsequently detached using a trypsin-ethylenediaminetetraacetic acid (EDTA) solution (0.5% porcine trypsin and 0.2% EDTA in PBS; Sigma-Aldrich, St. Louis, MO, USA) along with 0.2% Triton X-100. The bacterial cell mixture was diluted and plated onto LB plates to determine the number of adhered bacteria (Hawdon et al., 2010). In a parallel experiment, DNA was extracted from the cells by using the EZNA bacterial DNA isolation kit (Omega), according to manufacturer protocols. The samples were subjected to quantitative real-time polymerase chain reaction (PCR) in a 20-µL (*S. aureus*) or 25-µL (*P. aeruginosa*) reaction mixture, using specific primers and amplification programs, as summarized in Table 1.

Table 1. Primers and amplification program for quantitative real-time polymerase chain reaction analysis.

Pathogens and controls	Sequence (5' - 3')	Amplification program	References
Staphylococcus aureus	AATCTTTGTCGG TACACGATATTCTTCACG CGTAATGAGATTTCAGTAGATAATACA ACA	96°C for 3 min 30 or 40 cycles of denaturation at 95°C for 1 s and annealing-extension at 55°C for 30 s	Martineau et al., 1996
Pseudomonas aeruginosa	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	Initial denaturation: 95°C for 2 min Amplification: 25 cycles of 94°C for 20 s, 58°C for 20 s, and 72°C for 40 s Final extension: 72°C for 1 min	Anzai et al., 2000
TNF-α	ATGAGCACTGAAAGCATGATCCGG GCAATGATCCCAAAGTAGACCTGCCC	Initial denaturation: 94°C for 2 min Amplification: 35 cycles of 94°C for 30 s, 60°C for 45 s, and72°C for 60 s Final elongation: 72°C for 10 min	-
Beta actin	TGGCATTGTTACCAACTGGGACGA GCTTCTCTTTGATGTCACGCACG	Initial denaturation: 94°C for 2 min Amplification: 35 cycles of 94°C for 30 s, 60°C for 45 s, and 72°C for 60 s Elongation: 72 °C for 10 min	-

RNA extraction and reverse transcriptase-PCR (RT-PCR)

Total RNA was extracted from the enterocytes by using RNAiso plus, as described by Schnupf and Sansonetti (2012). The RNA concentration was determined using NanoDrop, and cDNA was synthesized using a cDNA kit (Transgene), according to the manufacturer protocols. The mRNA expression of tumor necrosis factor alpha (TNF-α) was determined by RT-PCR, using specific primers and amplification program, as detailed in Table 1.

Epifluorescence and confocal laser scanning microscopy

The bacterial adherence and colonization was established on a 6-well plate containing a cover slip for 48 h, using the protocol described above. Wells containing un-inoculated broth were utilized as the negative control. Following incubation, the cover slips were aseptically removed, washed thrice with 0.05 M phosphate buffer (pH 7.4), and immersed twice in 1 mL PBS for 5 min. The samples were stained with 0.5% w/v fluorescein isothiocyanate (FITC) stock solution in 1 mL PBS (5 µL) in the dark, washed with PBS and fixed with 4% paraformaldehyde for 1 h at ambient temperature (Hamanaka et al., 2001). In a parallel experiment, the cover slips were also stained

with 1% crystal violet (Thenmozhi et al., 2009) and 0.025% calcofluor white M2R (Sigma-Aldrich), which binds to beta-linked polysaccharides (Maeda and Ishida, 1967), for 1 min in the dark. The cover slips were observed and analyzed under a fluorescence microscope (IX71; Olympus, Tokyo, Japan) and a confocal laser scanning microscope (CLSM; SPS II; Leica, Wetzlar, Germany). This experiment was performed in duplicate.

Effect of CFS derived from lactobacilli on pathogenic extracellular polymeric substance (EPS)

A modified Congo red binding assay, described previously by Lee et al. (2007), was used to assess the effect of CFS on pathogenic exopolysaccharide. The bacterial cells were collected after centrifugation, and resuspended in 1 mL LB broth containing Congo red (40 µg/mL) and 100 µL CFS derived from lactobacilli. LB broth containing Congo red served as the negative control. All tubes were incubated at 37°C under agitation for 2 h. The bacterial cells stained with Congo red were removed by centrifugation, and the amount of Congo red dye remaining in the supernatant was quantified by measurement of the OD at 490 nm (OD_{490}).

Effect of CFS derived from lactobacilli on pathogenic cell surface hydrophobicity

The effect of CFS derived from lactobacilli on pathogenic cell surface hydrophobicity was measured with the microbial adhesion to hydrocarbon (MATH) assay, using a hydrocarbon solvent, as described in a previous study (Rosenberg et al., 1980).

Effect of CFS derived from lactobacilli on pathogenic viability

Live and dead cells were observed using a LIVE/DEAD *Bac*Light bacterial viability kit (Invitrogen), according to protocols detailed by the manufacturer. Briefly, 3.34 mM SYTO 9 nucleic acid stain and 30 mM propidium iodide (1.5 µL each) were added to all the tubes; the tubes were incubated for 15 min in the dark at 37°C. Following incubation, the samples (10 µL each) were placed on clean glass slides for microscopic observation. The bacteria were quantified by the plate count method in parallel.

Determination of inhibitory substances

The inhibitory metabolites present in CFS derived from lactobacilli were identified using the well diffusion assay. In addition, H_2O_2-mediated killing by lactobacilli was assessed on MRS agar supplemented with 0.25 mg/mL 3,3',5,5'-tetramethylbenzidine dihydrochloride hydrate and 0.01 mg/mL horseradish peroxidase (Sigma-Aldrich), respectively, as described by Song et al. (1999). Bacterial cultures were streaked as a thick linear inoculum onto the agar surface and incubated anaerobically for 48 h at 37°C. Following incubation, the plates were exposed to ambient atmosphere for 2 h. The development of a blue color was indicative of peroxide production.

Statistical analysis

The obtained results were statistically analyzed using Student's *t*-test, on the SPSS v.11.5 software platform (SPSS Inc., Chicago, IL, USA). P-values < 0.05 were considered to indicate statistical significance.

RESULTS

Effect of lactobacilli on human intestinal cell infection

The effect of whole cells and cell-free supernatant of lactobacilli on enterocyte infection caused by *S. aureus* and *P. aeruginosa* was assessed by three methods (pre-exposure, co-exposure, and post-exposure). The simultaneous application of whole cells and CFS significantly ($P < 0.05$) reduced the adherence of pathogens and development of infection; however, this effect was not observed when the lactobacilli were added after or prior to the infection ($P > 0.05$). This indicated a competitive mechanism (Figure 1). In addition, the *TNF-α* mRNA expression (determined by RT-PCR) indicated that the lactic acid bacteria reduced the enterocyte infection (Figure 2).

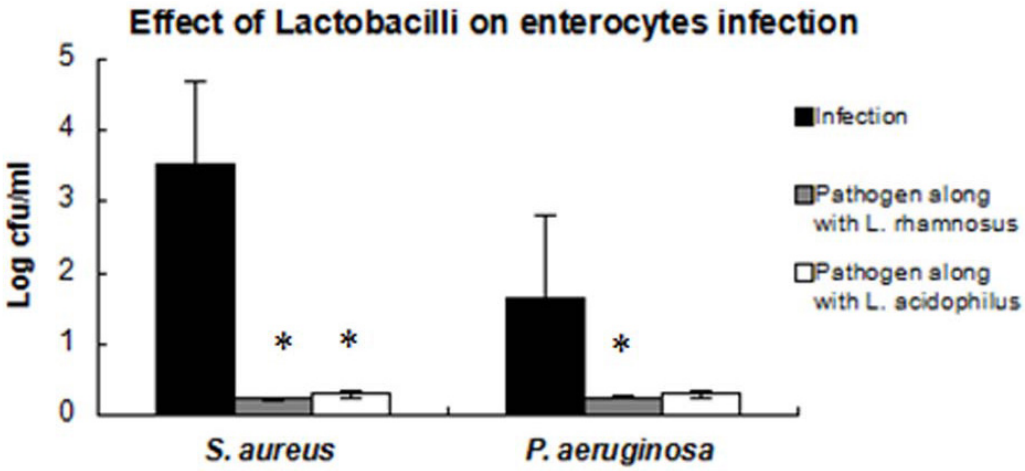

Figure 1. Effect of lactobacilli on enterocyte infection.

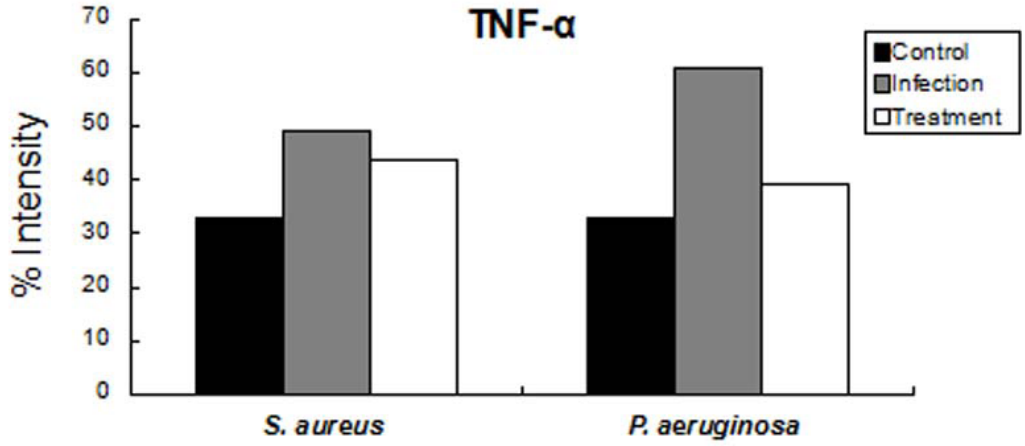

Figure 2. TNF-α mRNA expression.

Real-time RT-PCR quantification

The number of *S. aureus* DNA copies was reduced in the presence of *L. acidophilus* ($P < 0.05$); however, the co-exposure of *P. aeruginosa* and *L. acidophilus* failed to demonstrate any significant changes ($P > 0.05$), as summarized in Table 2. Quantitative analysis could not be performed using *L. rhamnosus*.

Table 2. Results of the quantitative real-time polymerase chain reaction (PCR) analysis (Means ± standard deviation).

Pathogens	Infected LoVo cells	Infected LoVo cells with *Lactobacillus acidophilus* cells	P values
Pseudomonas aeruginosa	17.815 ± 1.08	17.605 ± 0.91	P > 0.05
Staphylococcus aureus	3.899 ± 0.27	1.598 ± 0.40	P < 0.05

Data are reported as the average estimate of logarithms of the PCR target genetic amplicon copy numbers. P value < 0.05 (t-test) was considered (statistically) significantly different.

Epifluorescence and CLSM

The adherence of pathogens in the presence of CFS derived from lactobacilli was visualized by staining with FITC, crystal violet, and calcofluor white (Figures 3, 4, 5, and 6). FITC binds nonspecifically to the cell wall proteins, whereas calcofluor white binds to the beta-linked polysaccharides that aid in bacterial attachment. As expected (based on previously obtained results), CFS derived from lactobacilli reduced the adhesion of pathogenic bacteria to abiotic surface.

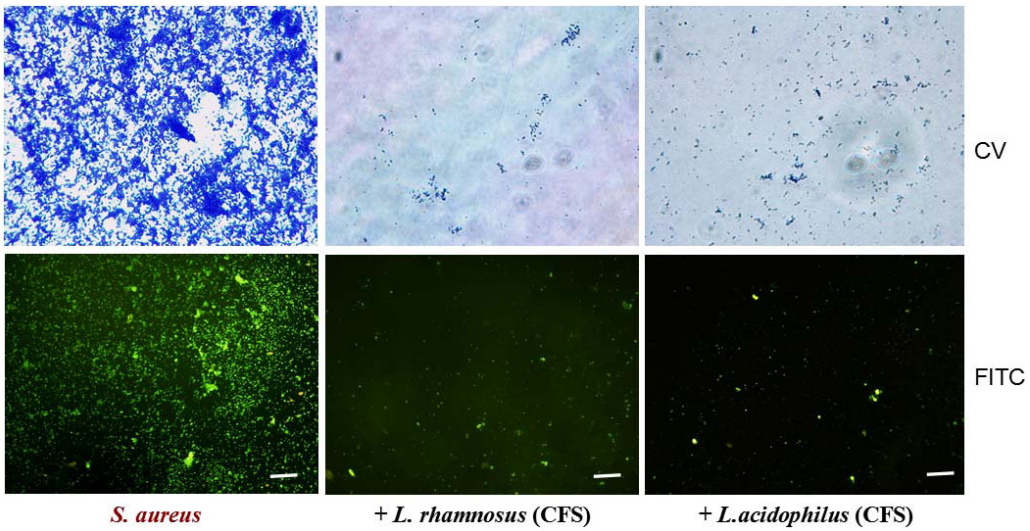

Figure 3. Adherence capacity of antibiotic-resistant *Staphylococcus aureus*. The adherence capacity of antibiotic-resistant *S. aureus* in the absence (control) and presence (+) of cell-free supernatant (CFS) derived from lactobacilli. Error bars, 100 µm.

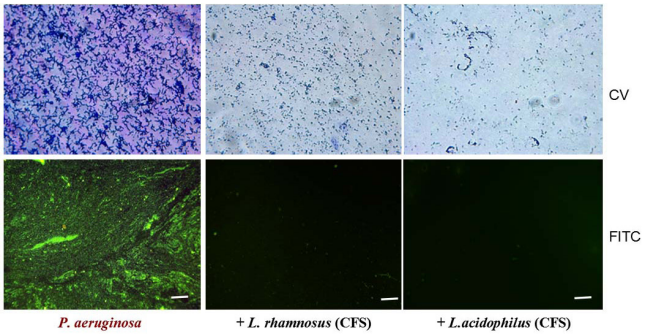

Figure 4. Adherence capacity of antibiotic-resistant *Pseudomonas aeruginosa*. The adherence capacity of pathogenic, antibiotic-resistant *P. aeruginosa* in the absence (control) and presence (+) of CFS derived from lactobacilli. Error bars, 100 µm

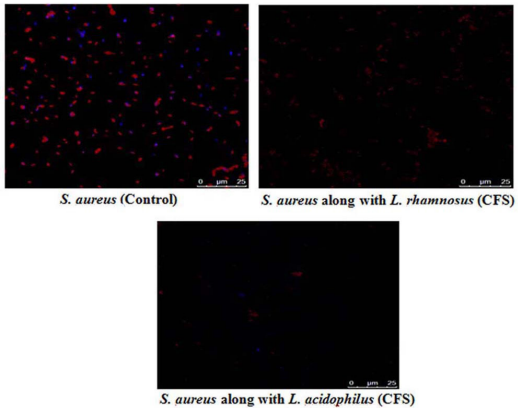

Figure 5. Effect of CFS derived from lactobacilli on the extracellular polymeric substance (EPS) of *S. aureus*. The confocal laser scanning microscopic (CLSM) analysis of EPS, stained with calcofluor white (blue), revealed the effect of CFS derived from lactobacilli on *S. aureus* EPS. Nucleic acid staining was achieved using ethidium bromide (red).

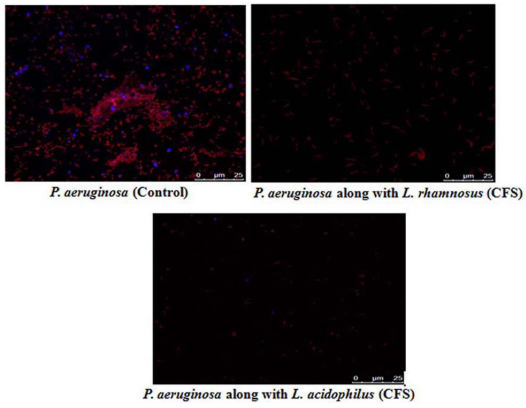

Figure 6. Effect of CFS derived from lactobacilli on the EPS of *P. aeruginosa*. CLSM analysis of EPS, stained with calcofluor white (blue), revealed the effect of CFS derived from lactobacilli on *P. aeruginosa* EPS. Nucleic acid staining was achieved using ethidium bromide (red).

Effect of CFS derived from lactobacilli on pathogenic extracellular polymeric substance

Bacterial EPS is the major constituent of biofilm matrix, which allows the bacteria to adhere and form a stable biofilm. The Congo red dye is used to target extracellular polymeric substances. Figure 7 indicated that CFS derived from lactobacilli decreased the ability of pathogens to bind to Congo red. This result supported the theory that CFS interferes with pathogen colonization by affecting the cell surface attachment of the pathogen to the substratum.

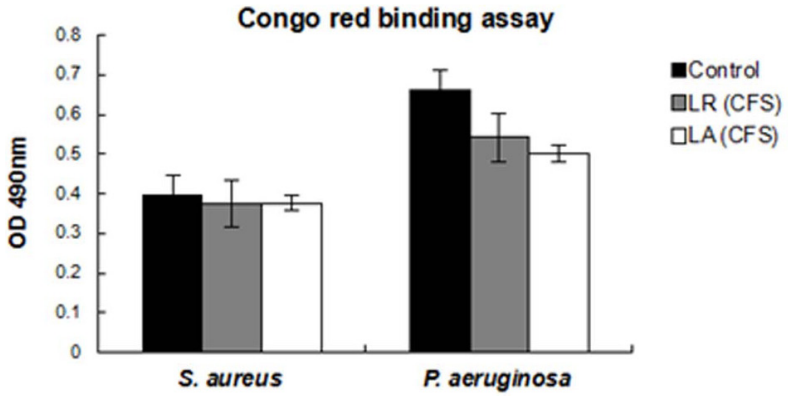

Figure 7. Congo red binding assay. LR, *Lactobacillus rhamnosus*; LA, *Lactobacillus acidophilus*.

Effect of CFS derived from lactobacilli on cell surface hydrophobicity of pathogens

We also attempted to determine the effect of CFS derived from lactobacilli on the surface hydrophobicity of pathogens. Figure 8 indicates that CFS derived from lactobacilli interferes with the surface hydrophobicity of pathogens. Alterations in the surface hydrophobicity result in non-attachment of the pathogens to the substratum; this affects colony formation, and ultimately, the host infection capacity of the pathogens.

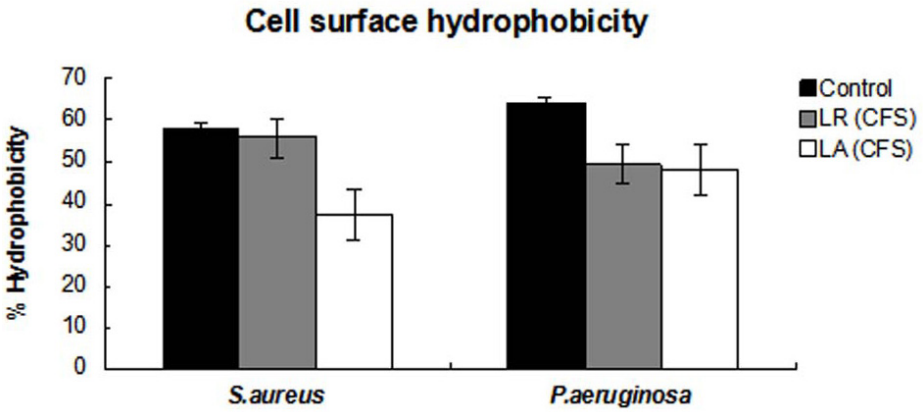

Figure 8. Effect of lactobacilli on pathogenic cell surface hydrophobicity. LR, *Lactobacillus rhamnosus*; LA, *Lactobacillus acidophilus*.

Effect of CFS derived from lactobacilli on the viability of *S. aureus* and *P. aeruginosa*

Thus far, we have demonstrated that CFS derived from lactobacilli inhibited enterocyte infection. In the next experiment, we investigated the effect of CFS on the viability of pathogens. The results of the plate count assay and Live/Dead *Bac*Light bacterial viability staining revealed the presence of some inhibitory substances in CFS derived from lactobacilli (neutral). *L. acidophilus*-derived CFS showed the maximum bactericidal activity, as depicted in Figures 9, 10, and 11.

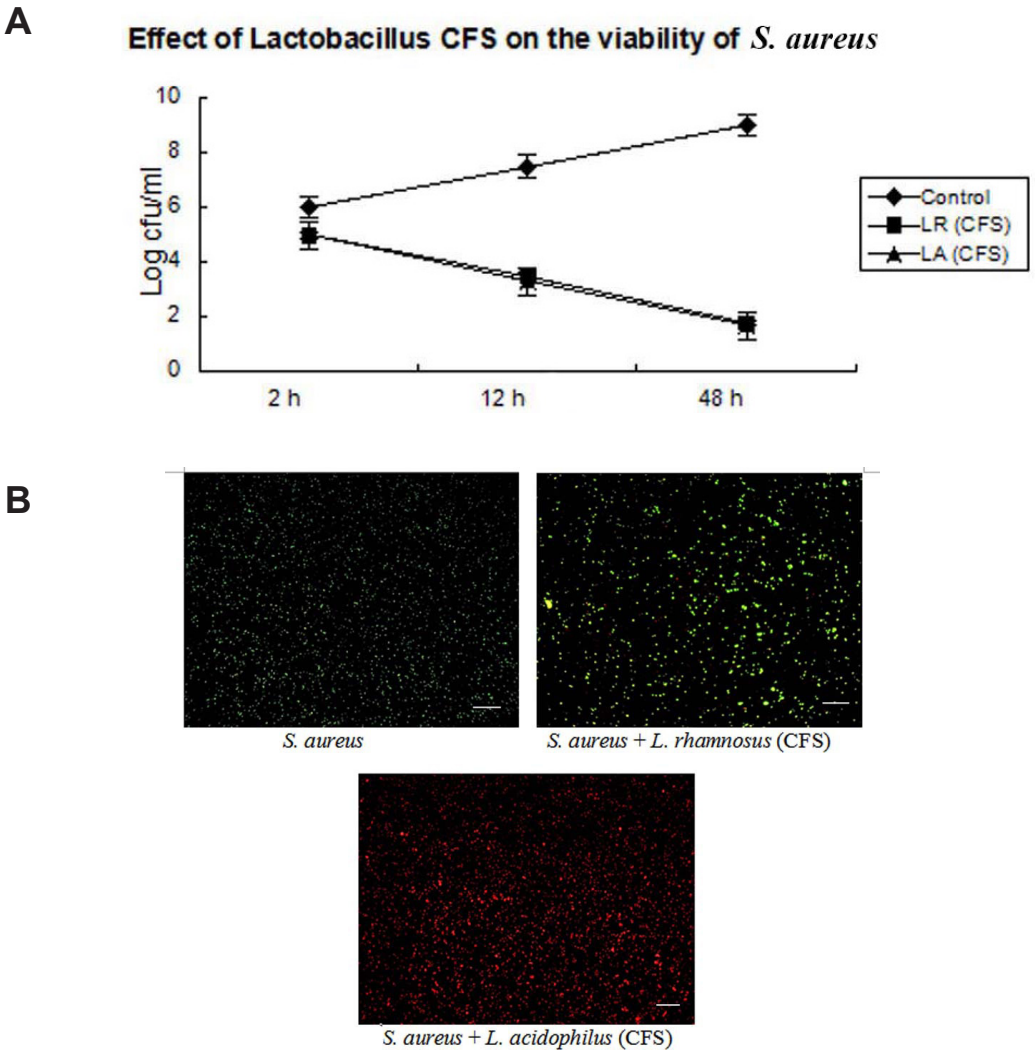

Figure 9. Effect of CFS derived from lactobacilli on *S. aureus* cell viability. The viability of pathogens was analyzed by the (**A**) plate count method and (**B**) by using the LIVE/DEAD *Bac*Light bacterial viability staining kit. Propidium iodide was used to stain the dead cells (red), while SYTO 9 stained the live cells (green). *S. aureus*, S; *L. rhamnosus*, LR; *L. acidophilus*, LA. Error bars, 100 µm.

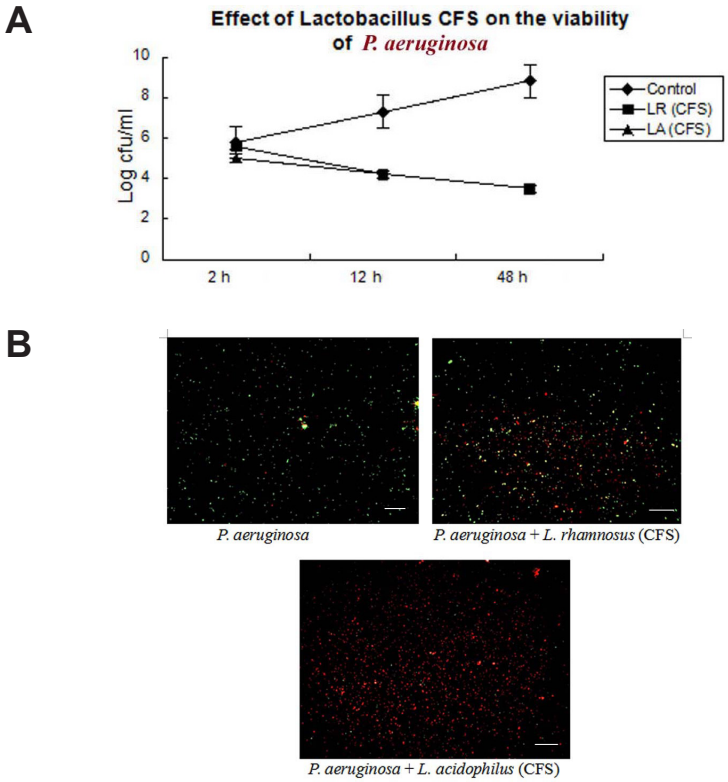

Figure 10. Effect of CFS derived from lactobacilli on *P. aeruginosa* cell viability. The viability of pathogens was analyzed by the (**A**) plate count method and (**B**) by using the LIVE/DEAD *Bac*Light bacterial viability staining kit. Propidium iodide stained the dead cells (red), while SYTO 9 stained the live cells (green). *P. aeruginosa*, P; *L. rhamnosus*, LR; *L. acidophilus*, LA. Error bars, 100 µm.

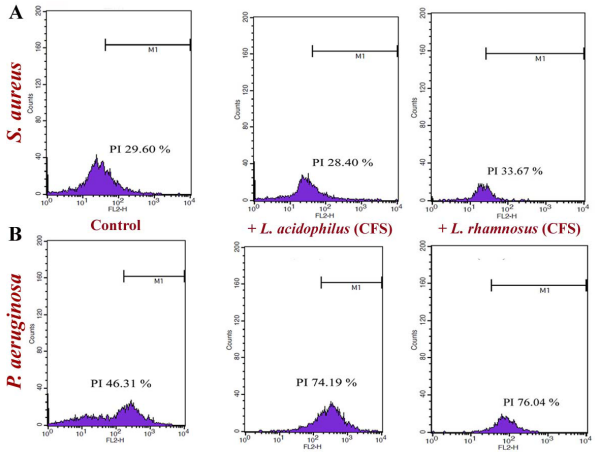

Figure 11. Analysis of bacterial cultures using *Bac*Light bacterial viability staining. *S. aureus* (Panel A) and *P. aeruginosa* (Panel B) cells were stained with propidium iodide (PI) and analyzed by flow cytometry.

Determination of inhibitory substances present in CFS derived from lactobacilli

We attempted to identify the inhibitory substances present in CFS via a number of experiments, including the agar well diffusion and H_2O_2 production assays. Neither of the tested lactobacilli showed the presence of any bacteriocin-like substances or H_2O_2 mediated killing activity. The pH dependent activity of CFS was excluded using a neutral CFS in all aspects of the study. This indicates the effect of non-bacteriocin competitive inhibitory molecules (and not organic acids) present in the CFS on the adhesive capacity (to the host cells) and viability of pathogens.

DISCUSSION

More than 90% of all bacteria colonizing the body are found in the intestines, which is also an ideal site for efficient transmission of resistance genes. Administration of antimicrobial agents greatly disrupts the activity of the intestinal microbiota; in addition, there is always a chance that the patient's intestine might be colonized by resistant organisms, resulting in the development of serious infections. Antibiotic-resistant *S. aureus* and *P. aeruginosa* are opportunistic pathogens with the ability to promote intestinal infections. The clinical implications of intestinal colonization by *S. aureus* and *P. aeruginosa* are still relatively undefined. In this study, we have examined the effects of lactobacilli on enteric infections caused by Gram-positive *S. aureus* and Gram-negative *P. aeruginosa.*

Lactic acid bacteria competitively inhibit the development of enterocyte infection by *S. aureus* and *P. aeruginosa*. Host enterocytes simultaneously exposed to lactobacilli or their CFS and pathogens were protected from the effects of the pathogen. This phenomenon was further confirmed by fluorescence microscopic observations, which revealed the competitive inhibition of pathogenic adherence. FITC binds non-specifically to the cell wall proteins (Brul et al., 1997). Competitive exclusion resulting from the inhibition of adhesion has been extensively studied using cultured human intestinal cells. Some studies have shown that competitive inhibition is often strain dependent; on the other hand, displacement is generally not as effective, or requires a longer time to occur (Lee et al., 2003; Zarate and Nader-Macias, 2006).

Extracellular polymeric substances and cell surface hydrophobicity aid in the colonization and biofilm formation of bacteria on the host cell surface. In this study, the CFS derived from lactobacilli did not allow the pathogens to adhere and infect intestinal cells; therefore, we attempted to determine its effect on pathogenic EPS and cell surface hydrophobicity. Congo red binds to exopolysaccharides involved in cell adhesion and biofilm formation (Weiner et al., 1995). The results of both experiments revealed that CFS derived from lactobacilli evidently affected EPS synthesis, thereby interfering with the colonization of pathogens by affecting their attachment to the substratum. This was confirmed by the results of the CLSM analysis. The cell surface hydrophobicity of microorganisms plays an important role in bacterium-host cell interactions (Swiatlo et al., 2002). Any strategy responsible for reducing the cell surface hydrophobicity and EPS synthesis may affect the development of infection, making the cells more susceptible to antibiotics. A previous study also reported that some plant extracts inhibit the biofilm-forming capacity of Gram-positive and Gram-negative bacteria by interfering with their hydrophobicity (Annuk et al., 1999; Nostro et al., 2004; Razak et al., 2006).

P. aeruginosa and *S. aureus* infections are difficult to eradicate because of their resistance to many antibiotics. CFS derived from lactobacilli inhibits the development of infections by affecting the attachment of pathogens, in addition to influencing the viability of pathogens. The plate count method and the *Bac*Light bacterial viability test revealed the bactericidal effect of neutral CFS. We

conducted a number of experiments to identify the inhibitory substances present in CFS derived from lactobacilli. These experiments demonstrated that inhibitory substances other than organic acids compete with the pathogens for adhesion to the host cells, thereby compromising their cellular viability. This bactericidal activity was attributed to a yet unidentified non-bacteriocin-like substance, which functioned independent of organic acids and H_20_2 production. In contrast, an earlier study attributed the inhibition of some Gram-positive and Gram-negative bacteria by *L. acidophilus* BGRA43 to lactic acid production, rather than to hydrogen peroxide or bacteriocin activity (Banina et al., 1998). The reduction in pH and accumulation of ionized organic acids within bacteria leads to its death (Reid et al., 2003). The lactic acid bacteria countered (and inhibited) pathogenic infection by using bactericidal compounds other than organic acids, as only neutral CFS was used in this study. Therefore, it can be hypothesized that competition for the binding site is a major mechanism through which lactic acid bacteria antagonize *S. aureus* and *P. aeruginosa.* Lactic acid bacteria counteract the adhesive and invasive function of pathogenic bacteria by causing a nonspecific steric hindrance action on the pathogenic receptors (Bernet et al., 1993; Bernet et al., 1994), and by producing inhibitory metabolites. Coconnier-Polter et al. (2005) have previously reported that the CFS of *L. acidophilus* could potentially decrease the intracellular ATP content in *S. enterica* SL1344. Our results are also consistent with those obtained by Forestier et al. (2001), who demonstrated (in an *in vitro* study) that *L. casei rhamnosus* 35 inhibits the growth of both Gram-positive cocci and Gram-negative bacilli, including *P. aeruginosa*.

S. aureus and *P. aeruginosa* are common colonizers of the human intestine in hospitalized or immunosuppressed patients or those treated with antibiotics, leading to enteric infections. The results may serve as a theoretical basis for the development of anti-*P. aeruginosa* and -*S. aureus* therapeutic regimens. Lactic acid bacteria inhibited the pathogenic activity by interfering with the production of extracellular polymeric substance and the cell surface hydrophobicity of the pathogen, which in turn affected the colonization and infection capacity of the pathogens. Moreover, this inhibition was independent of the activity of *Lactobacillus*-produced organic acids. However, additional studies are required to evaluate the possible *in vivo* effects of whole cells and cell-free supernatants of lactobacilli on opportunistic enteric infections caused by antibiotic-resistant *P. aeruginosa* and *S. aureus*.

Conflicts of interest

The authors declare no conflict of interest.

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