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Assessment of synergistic combination potential of probiotic and bacteriophage against antibiotic-resistant *Staphylococcus aureus* exposed to simulated intestinal conditions

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Abstract This study was designed to evaluate the combined effect of probiotic *Lactobacillus rhamnosus* and bacteriophage SA11 on the control of antibiotic-sensitive *Staphylococcus aureus* (ASSA) and antibiotic-resistant *S. aureus* (ARSA) under the simulated intestinal conditions. The survivability of ASSA and ARSA were determined in the simulated phosphate-buffered saline (PBS)-, trypticase soy broth (TSB)-, and milk-based gastric juices adjusted to pH 2.0, 3.0, and 5.0 at 37 °C for 30 min. The inhibitory effect of bacteriophage SA11 and probiotic on the growth of ASSA and ARSA was evaluated in the simulated intestinal juices at 37 °C for 20 h. The least reductions in the numbers of ASSA and ARSA were observed in the milk-based gastric juices at pH 2.0 (<1 log). No significant changes in the teichoic acid-mediated sliding motility were observed for ASSA and ARSA after 30-min exposure to the simulated gastric juices (pH 2.0, 3.0, and 5.0), responsible for the enhanced bacterial attachment to the epithelial cells. The bacteriophage SA11 was stable down to pH 5.0 and up to 0.06 % bile salts. The bacteriophage SA11 combined with probiotic effectively inhibited the growth of ASSA and ARSA in the simulated intestinal conditions, showing more than 4 log reduction. The relative expression levels of adhesion-related genes (*clfA*, *eno*, and *fnbA*) and efflux-related genes (*mdeA*, *norB*, and *norC*) were less decreased in ARSA than in ASSA after exposure to the simulated gastrointestinal conditions. These results might shed light on the application of bacteriophage to control the ingested antibiotic-resistant foodborne pathogens in the intestinal tract.

Keywords Probiotic · Bacteriophage · Antibiotic resistance · Bile salt · Sliding motility · Gastrointestinal tract

Introduction

Over the last decade, the overuse and misuse of antibiotics have mainly resulted in the rapid increase in antibiotic-resistant bacteria, which can eventually lead to serious health problems. Long-term antibiotic use can cause an imbalance of indigenous intestinal microflora and furthermore result in a selective pressure towards stress-resistant bacteria, known as cross-resistance (Mathur and Singh 2005; Hammad and Shimamoto 2010). Ingested antibiotic-resistant pathogenic bacteria encounter mechanical, chemical, and biological barriers, including acidic pH, proteolytic enzymes, detergents, peristaltic action, and indigenous enteric microflora, during the gastrointestinal transit (Bourlioux et al. 2003). In order to pass through the stomach and reach the large intestine alive, however, bacteria have evolved several survival strategies such as acid tolerance response (ATR) system and bile resistance mechanism (Gunn 2000; Foster 2001). In addition, food matrices can cause change in gastric acidity and provide protective effect to ingested bacterial pathogens (Barmpalia-Davis et al. 2008; Koseki et al. 2011). The inhibitory effect of gastrointestinal fluids can thus be overestimated, while the viability of ingested antibiotic-resistant bacterial pathogens can possibly be underestimated in the gastrointestinal tract (GIT) (Smith 2003). Therefore, it is necessary to evaluate

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the fate of bacterial pathogens exposed to the GI conditions and develop novel preventive and therapeutic approaches as antibiotic alternatives.

Probiotics, "the live feed supplements which beneficially affect the host animal by improving its intestinal microbial balance", have been well known as holistic approach in the GIT to provide health benefits, including the modulation of intestinal microflora, the competitive exclusion of pathogens, and stimulation of immune system (Fuller 1989; Servin and Coconnier 2003; Collado et al. 2007; Woo and Ahn 2013). Recently, bacteriophages control has received much research attention to preventing and treating infectious diseases due to their specificity to bacterial hosts, effectiveness against antibiotic-resistant bacteria, and no adverse side effects (Matsuzaki et al. 2005; Atterbury et al. 2007; Bardina et al. 2012). Since the inhibitory mechanisms of bacteriophages against the host bacteria are different from the modes of action of antibiotics, the use of bacteriophages has the potential for controlling antibiotic-resistant pathogens (Matsuzaki et al. 2005). However, little is known about the combined effect of probiotic and bacteriophage on the inhibition of antibiotic-resistant bacterial pathogens exposed to the simulated intestinal conditions. *Staphylococcus aureus*, an opportunistic pathogen, is a frequent cause of nosocomial infections. Many studies have been reported that *S. aureus* can colonize the intestinal tract through hospitalized patients (Vesterlund et al. 2006; Bhalla et al. 2007). The production of staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1) plays an important role in the pathogenesis of *S. aureus* infections, leading to foodborne disease outbreaks and intestinal colonization (Bhalla et al. 2007; Pereira et al. 2009). Therefore, the objectives of this study were to evaluate the survival of antibiotic-resistant *S. aureus* (ARSA) in the different medium-based solutions adjusted to pH 2.0, 3.0, and 5.0 during the simulated gastric transit and also assess the possibility of using probiotic combined with bacteriophage as an antibiotic alternative as evaluated by the susceptibility of ARSA to the simulated intestinal conditions.

Materials and methods

Bacterial strains and culture conditions

Strains of *Staphylococcus aureus* ATCC 13301 and *S. aureus* CCARM 3080 obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and Culture Collection of Antibiotic Resistant Microbes (CCARM, Seoul, Korea), respectively, were cultured in trypticase soy broth (TSB) (BD, Becton, Dickinson and Co., Sparks, MD, USA) at 37 °C for 24 h. According to the predetermined antibiotic susceptibilities (data not shown), *S. aureus* ATCC

13301 and *S. aureus* CCARM 3080 were assigned to ASSA and ARSA, respectively. Strain of *Lactobacillus rhamnosus* GG was kindly provided by Dr. Azlin Mustapha of the Department of Food Science at the University of Missouri (Columbia, MO, USA). Probiotic strain was grown anaerobically in de Man Rogosa Sharpe broth (MRS; Difco, BD) supplemented with 0.05 % L-cysteine hydrochloride at 37 °C for 24 h. All strains were harvested by centrifugation at $5,000 \times g$ for 10 min at 4 °C. The harvested cells were washed twice with sterile 0.1 % peptone water.

Bacteriophage propagation

Staphylococcus bacteriophage SA11 purchased from the Bacteriophage Bank at Hankuk University of Foreign Studies (Yongin, Gyeonggi, Korea) belongs to the Siphoviridae family, containing a linear, double-stranded DNA genome (Kim and Myung 2012). The bacteriophage SA11 was propagated at 37 °C for 24 h in TSB containing *S. aureus* ATCC 13301 as an appropriate host strain. After the bacteriophage propagation, the supernatant obtained by centrifugation at $5,000 \times g$ for 10 min was filtered through a 0.2-μm filter to eliminate residual bacterial lysates. The titre of bacteriophage SA11 was determined by using soft agar overlay method. The filtrate was serially (1:10) diluted with phosphate-buffered saline (PBS; pH 7.4). Each decimal dilution (100 μl) was gently mixed with the host cells (10^8 CFU/ml) in 0.5 % TSB soft agar and then immediately poured onto the prewarmed base agar. The plates were incubated at 37 °C for 24 h to quantify the bacteriophage expressed as plaque-forming unit (PFU).

Simulated gastric transit assay

The simulated gastric juice was prepared by dissolving 0.3 % pepsin in basal PBS, TSB, or milk and adjusted to pH 2.0, 3.0, and 5.0 by using 6 N hydrochloric acid (HCl). Approximately 10^7 CFU/ml of *S. aureus* cells were inoculated in different media (PBS, TSB, or milk) of varying pH levels and then incubated anaerobically for 30 min at 37 °C in a GasPak anaerobic system (BBL, Cockeysville, MD, USA) with Anaerogen (Oxoid, ltd, Hampshire, UK).

Bacterial sliding motility assay

The bacterial sliding motility was measured according to the sliding motility assay (Kaito and Sekimizu 2007). *S. aureus* cells exposed to the PBS- or TSB-based gastric juices at 37 °C for 30 min were centrifuged at $3,000 \times g$ for 20 min, rinsed with PBS, and adjusted to 1×10^8 CFU/ml. Each culture (2 μl) was stabbed into the centre of soft agar plates (0.24 % agar). The plates were incubated at 37 °C for 24 h. The diameters of circle zone on the plates were

measured using a metric ruler. The sliding motility for each treatment was expressed as per cent compared to the control.

Acid and bile susceptibility assay

The susceptibility of bacteriophage SA11 to acid and bile salts was evaluated in TSB adjusted to pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 with 6 N HCl and serially diluted (1:2) at concentration of 0.45 % bile salts. Approximately 10^7 PFU/ml of bacteriophage SA11 was inoculated in the prepared TSB at different levels of pHs and bile salts and then incubated at 37 °C for 20 h anaerobically. The numbers of bacteriophage SA11 were enumerated to evaluate the acid and bile susceptibilities.

Simulated intestinal transit assay

For the intestinal transit assay, the simulated intestinal juice was prepared by suspending 0.03 % bile salt in TSB adjusted to pH 8.0 with 6 N NaOH (TSB-8). Approximately 10^7 CFU/ml of *S. aureus* cells were inoculated anaerobically in the simulated intestinal juice with *L. rhamnosus* and/or bacteriophage SA11 and incubated at 37 °C for 20 h. Treatments included TSB-8, *L. rhamnosus* (LR), bacteriophage SA11 (SA11), and *L. rhamnosus* and SA11 (LR+SA11).

RNA extraction and cDNA synthesis

RNA was extracted according to the protocol of RNeasy Protect Bacteria Mini kit (Qiagen, Hilden, Germany). The cells (0.5 ml) exposed to the simulated gastric (TSB-3) and intestinal (TSB-8, LR, SA11, LR+SA11) juices were mixed with 1 ml of RNA protect Bacteria Reagent and centrifuged at 5,000×*g* for 10 min. The harvested cells were lysed with a buffer containing lysozyme. The lysates were mixed with ethanol to extract RNA using an RNeasy mini column. The genomic DNA was removed by a Wipe buffer. The cDNA synthesis was carried out according to the QuantiTech reverse transcription procedure (Qiagen). The RNA extracts were mixed with a master mixture of reverse transcriptase, RT buffer, and RT primer mix and then incubated at 42 °C for 15 min followed by 95 °C for 3 min to inactivate the reverse transcriptase. The cDNA synthesized was used as a template for quantitative RT-PCR assay.

Quantitative RT-PCR assay

The reaction mixture (20 μl) containing 10 μl of 2 × QuantiTect SYBR Green PCR Master, 2 μl of each primer, 2 μl of cDNA, and 4 μl of RNase-free water was amplified using an iCycler iQ™ system (Bio-Rad Laboratories, Hemel Hempstead, UK). The oligonucleotides (Erofins MWG Operon; Huntsville, AL, USA) used as primers of *S. aureus* are listed in Table 1. The PCR mixture was denatured at 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s, 55 °C for 20 s, and 72 °C for 15 s. The relative gene expression levels were estimated using the comparative method (Livak and Schmittgen 2001). The C_T values of target genes in *S. aureus* cells exposed to the simulated gastric and intestinal juices were compared to the C_T values obtained from the untreated cells (control). The 16S ribosomal RNA as the reference gene was used for target gene normalization.

Statistical analysis

All analyses were carried out in duplicate on three replicates. Data were analysed using the Statistical Analysis System software. The general linear model and least

Table 1 Primers used in quantitative RT-PCR analysis for *S. aureus*

Target mRNA	Molecular function	Primer sequence
16S rRNA	Reference gene	F: CATGCTGATCTACGATTACT R: CCATAAAGTTGTTCTCAGTT
clfA	Clumping factor A	F: ATTGGCGTGGCTTCAGTGCT R: CGTTTCTTCCGTAGTTGCATTTG
eno	Laminin-binding protein	F: ACGTGCAGCAGCTGACT R: CAACAGCATYCTTCAGTACCTTC
fnbA	Fibrinogen-binding protein A	F: CATAAATTGGGAGCAGCATCA R: ATCAGCAGCTGAATTCCCATT
mdeA	Multidrug efflux A	F: GTTTATGCGATTCGAATGGTTGGT R: AATTAATGCAGCTGTTCCGATAGA
norB	Efflux transporter protein	F: AGCGCGTTGTCTATCTTTCC R: GCAGGTGGTCTTGCTGATAA
norC	Efflux transporter protein	F: AATGGGTTCTAAGCGACCAA R ATACCTGAAGCAACGCCAAC

F and R indicate forward and reverse primers, respectively

significant difference (LSD) procedures were used to compare means. Significant mean differences were estimated by Fisher's LSD at $P < 0.05$.

Results and discussion

This study describes the inhibitory effect of probiotic combined with bacteriophage SA11 against ASSA and ARSA in the simulated intestinal condition. Relatively few studies have been conducted to assess the bacteriophage effect against ingested foodborne pathogens. Thus, this study is a new approach to investigate the potential of using bacteriophage in combination with probiotic function for controlling ingested antibiotic-resistant foodborne pathogens in the GIT.

Survival of *S. aureus* in the simulated gastric juices at various pHs

The acid tolerance of ASSA and ARSA was evaluated in the simulated PBS-, TSB-, and milk-based gastric juices adapted at pH 2.0, 3.0, and 5.0 (Fig. 1). The viabilities of ASSA and ARSA were not affected by pH 3.0 and 5.0, while those were significantly decreased depending on the composition of basic media and the physiological state of strains at pH 2.0. ASSA was more susceptible to pH 2.0 than ARSA in the simulated PBS- and TSB-based gastric juices. The numbers of ASSA were reduced by 2.8 and 6.0 log, respectively, in PBS- and TSB-based gastric juices. This suggests that antibiotic-resistant bacteria can induce adaptation to subsequent stress, known as cross-protection that describes the phenomenon in which mono-stress-adapted bacteria can easily acquire resistance to additional stresses (Greenacre et al. 2006; Xu et al. 2008). No significant difference was observed between the numbers of ASSA and ARSA in milk-based gastric juices adapted at pH 2.0, showing <1 log reduction. This result implies that food matrices can provide protective effect against acid stress under the gastric condition for ingested pathogenic bacteria, resulting in an increase in bacterial survivability (Zhu et al. 2006; Ceuppens et al. 2012). This protective effect is mainly due to the binding ability and buffering capacity of food components (Zhu et al. 2006; Barmpalia-Davis et al. 2008). Therefore, the surviving bacteria after gastric transit within food matrices can be released into the duodenum (pH 8) with high numbers, which undergo continuous multiplication and infectious process. The sliding abilities of ASSA and ARSA exposed to the simulated PBS- and TSB-based gastric juices at pH 3.0 (PBS-3 and TSB-3) and pH 5.0 (PBS-3 and TSB-3) were evaluated based on the colony-spreading force of growing cells (Fig. 2). Compared to the untreated
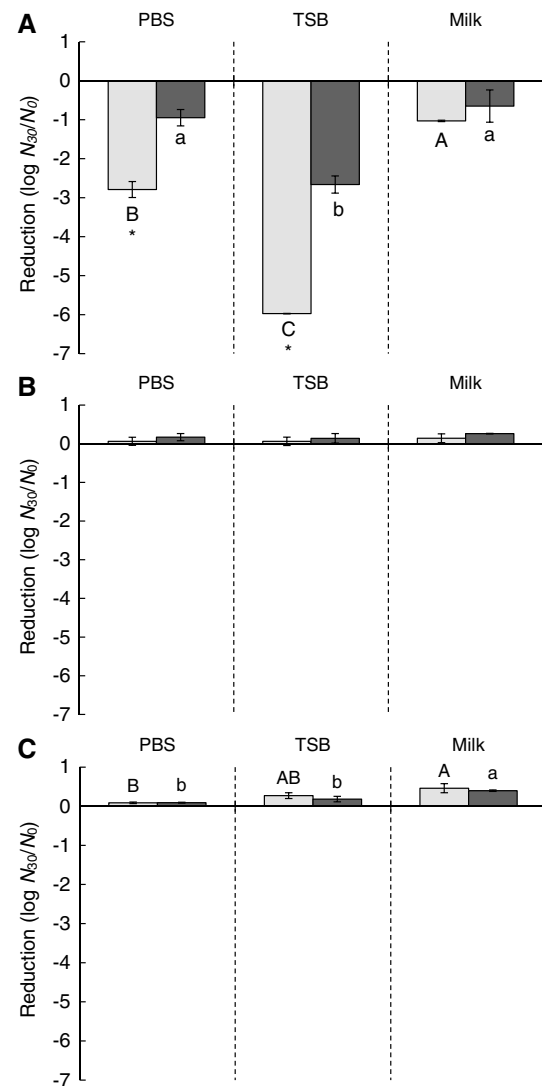

Fig. 1 Reduction (log N_{30}/N_0) of antibiotic-sensitive *S. aureus* (ASSA; *grey square*) and antibiotic-resistant *S. aureus* (ARSA; *filled square*) exposed to the PBS-, TSB-, and milk-based gastric juices adjusted to pH 2 (**a**), pH 3 (**b**), and pH 5 (**c**) at 37 °C for 30 min. *Bars with different uppercase letters* (**a–c**) and *lowercase letters* (**a–b**) are significantly different within ASSA (*grey square*) and ARSA (*filled square*), respectively, at $P < 0.05$. N_0 and N_{30} represent the inoculum level (7.12 log CFU/ml) and the bacterial count after incubation of 30 min, respectively. *Asterisk* (*) indicates the significant difference between ASSA (*grey square*) and ARSA (*filled square*)

control, the colony-spreading abilities of ASSA and ARSA were not significantly changed after exposure to PBS-3, TSB-3, PBS-3, and TSB-3, ranging 93–111 %. Teichoic acid-mediated sliding motility is mainly involved in the attachment of Gram-positive bacteria (Kaito and Sekimizu 2007). The observations suggest that ASSA and ARSA can enhance the abilities to attach to human epithelial cells, which plays an important role in pathogenesis (Kaito and Sekimizu 2007).
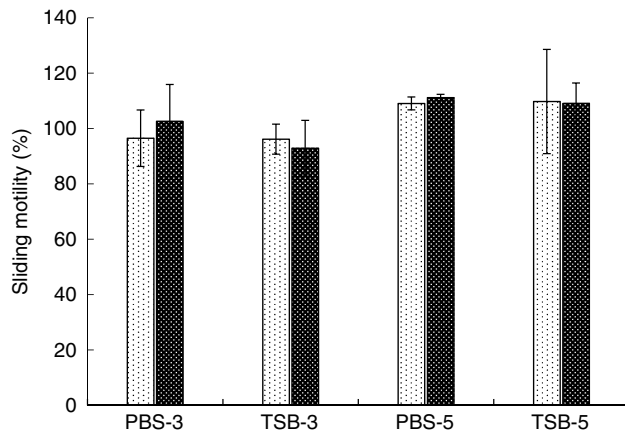
Fig. 2 Sliding motility (%) of antibiotic-sensitive *S. aureus* (ASSA; *white dotted bar*) and antibiotic-resistant *S. aureus* (ARSA; *dark dotted bar*) exposed to the PBS- and TSB-based gastric juices adjusted to pH 3 (PBS-3 and TSB-3) and pH 5 (PBS-5 and TSB-5) at 37 °C for 30 min. The untreated *S. aureus* was normalized to 100 %

Effect of pH and bile salts on the stability of bacteriophage SA11

The pH and bile salt susceptibilities of bacteriophage SA11 were evaluated as shown in Fig. 3. The viability of bacteriophage SA11 was significantly affected at pH below 4.0, showing the bacteriophage counts below the limit of detection (<1 log PFU/ml) (Fig. 3a). This is in good agreement with previous reports that bacteriophages are stable at a pH range of 5–8 (Chow and Rouf 1983). The stability of bacteriophage SA11 was decreased at pH 5.0 with increasing incubation time. The bacteriophage counts were 6.8, 6.5, and 5.6 log PFU/ml after 2, 10, and 20 h of incubation at pH 5.0. The numbers of bacteriophage remained high above pH 7.0 regardless of incubation time. The susceptibility of bacteriophage SA11 to bile salts was slightly decreased with increasing the concentration (Fig. 3b). No significant changes in the bacteriophage counts were observed until 0.06 % bile salts. The results imply that the bacteriophage SA11 is applicable for controlling *S. aureus* in the intestinal tract.

Viability of *S. aureus* in the simulated intestinal juices treated with probiotic and bacteriophage

The inhibitory effect of probiotic combined with bacteriophage SA11 on the growth of ASSA and ARSA was evaluated in the simulated intestinal juices (Fig. 4). The growth of ASSA and ARSA in the simulated intestinal condition was significantly inhibited by *L. rhamnosus* (LR), bacteriophage (SA11), and combination (LR + SA11) compared to the control. The number of ASSA was significantly
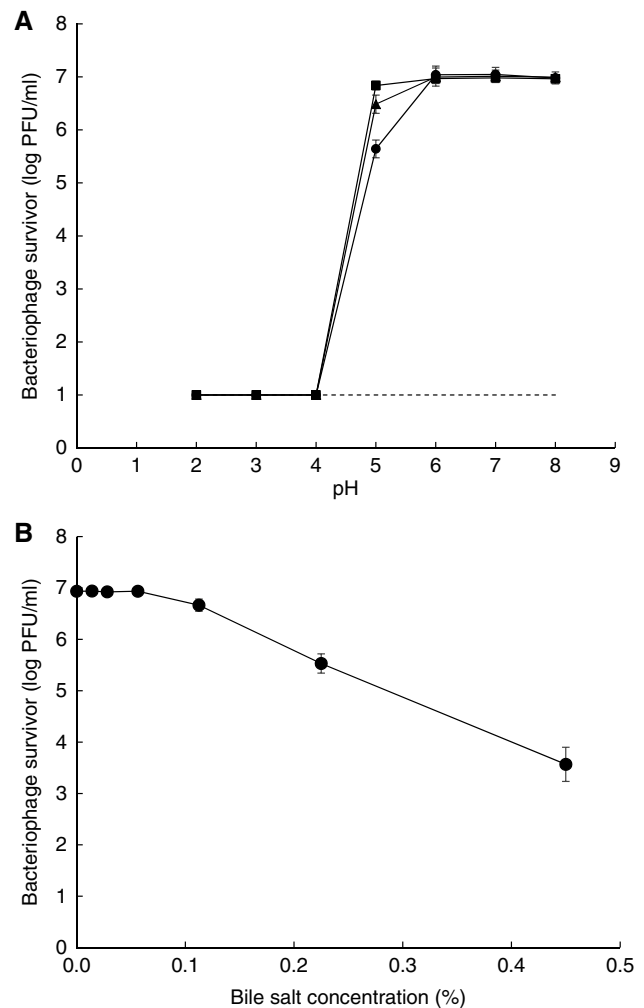

Fig. 3 Susceptibility of bacteriophage SA11 to pH (**a**) at 2-h (*filled square*), 10-h (*filled triangle*), and 20-h (*filled circle*) incubation and bile salts (**b**). The *dotted line* indicates the level of detection (1 log PFU/ml)

decreased to 3.5 log CFU/ml in the intestinal juice treated with bacteriophage SA11 when compared to ARSA (7.0 log CFU/ml) after 10 h of incubation (Fig. 4a). This might be due to the altered specific carbohydrate receptors on the surface of ARSA, leading to the decrease in bacteriophage adsorption (Muller-Merbacha et al. 2007; Bleackley et al. 2009). The inhibitory effect of LR, SA11, and LR plus SA11 was more noticeable against ASSA than ARSA after 20 h of incubation (Fig. 4b). This suggests that ARSA might induce cross-protective phenomenon in the simulated intestinal conditions (Greenacre et al. 2006). Bacteriophage SA11 effectively inhibited the growth of ASSA and ARSA after 10 h of incubation, while *L. rhamnosus* was effective against ASSA and ARSA after 20 h of incubation. This suggests that the lytic activity of bacteriophage SA11 was predominant at 10 h of incubation, while the probiotic activity was increased at 20 h. The growth
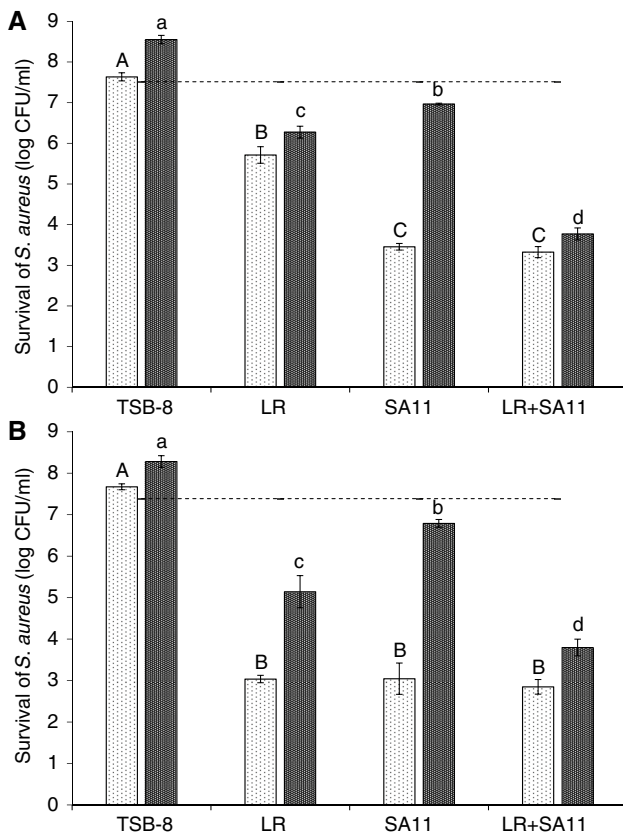
Fig. 4 Survival of antibiotic-sensitive *S. aureus* (ASSA; *white doted bar*) and antibiotic-resistant *S. aureus* (ARSA; *dark doted bar*) exposed to the simulated intestinal juices treated with *Lactobacillus rhamnosus* (LR; 7.32 log CFU/ml), bacteriophage (SA11; 7.07 log PFU/ml), and *L. rhamnosus* combined with bacteriophage (LR+SA11) at 37 °C for 10 h (**a**) and 20 h (**b**). The *dotted line* indicates the inoculum level of *S. aureus* (7.45 log CFU/ml). *Bars with different uppercase letters* (**a–c**) and *lowercase letters* (**a–d**) are significantly different within ASSA (*white doted bar*) and ARSA (*dark doted bar*), respectively, at *P* < 0.05

of probiotics produces metabolites such as organic acids, hydrogen peroxide, and bacteriocins, which are responsible for anti-pathogenic activity in the intestinal tract (Oelschlaeger 2010). The highest reductions in the numbers of ASSA and ARSA were observed at the combined treatment (LR+SA11), showing more than 4 log reductions at 10 and 20 h of incubation. Unlike ASSA, these results were not enough to clearly explain the combined effect of bacteriophage SA11 and *L. rhamnosus* against ARSA. Although further studies are required to elucidate the possible mechanisms of the combined effect of bacteriophage SA11 and *L. rhamnosus* in the simulated intestinal tract, this can be assumed that the reversible receptors and/or charge on the surface of ARSA was possibly altered by probiotic metabolites in the simulated intestinal conditions, resulting in an enhanced affinity between bacteriophages and host cells.

Gene expression patterns of ASSA and ARSA exposed to under simulated gastrointestinal conditions

The relative expression of adhesion-related genes (*clfA*, *eno*, and *fnbA*) and efflux-related genes (*mdeA*, *norB*, and *norC*) was observed in ASSA and ARSA exposed to the simulated gastric juice containing 0.3 % pepsin in TSB of pH 3.0 (TSB-3) for 30 min and the simulated intestinal juices containing 0.03 % bile salt in TSB of pH 8.0 (TSB-8) with *L. rhamnosus* (LR), bacteriophage SA11 (SA11), and *L. rhamnosus* and SA11 (LR+SA11) for 20 h (Fig. 5). Most genes were down-regulated in all treatments, while the genes, *clfA*, *fnbA*, and *norC*, were up-regulated in both ASSA and ARSA exposed to LR. The relative expression levels of *clfA* gene were increased to 1.8-fold in ASSA and 1.2-fold in ARSA when exposed to LA (Fig 5a). The *eno* and *fnbA* genes were up-regulated 1.5-fold and more than threefold in ARSA, respectively, after 20-h exposure to TSB-8 (Fig. 5b, c). The genes, *clfA*, *eno*, and *fnbA*, encode clumping factor, laminin-binding protein, and fibrinogen-binding protein, respectively, leading to increased staphylococcal virulence and resistance to host phagocytosis (O'Connell et al. 1998; Lei et al. 2011). The overexpression of *clfA*, *eno*, and *fnbA* is highly associated with the ability of *S. aureus* to form biofilm cells, adhere to epithelial cells, and evade the host immune system (Vancraeynest et al. 2004; Nemati et al. 2009; Stutz et al. 2011). The highest expression levels of *mdeA*, *norB*, and *norC* were observed in ARSA, showing 2.2-fold at LR, 0.3-fold at TSB-8, and 3.5-fold at LR, respectively (Fig. 5d, e, f). The expression of efflux-related genes (*mdeA*, *norB*, and *norC*) plays an important role in increasing antibiotic resistance of *S. aureus* (Huang et al. 2004; DeMarco et al. 2007; Truong-Bolduc et al. 2011). The relative expression levels of most genes were less decreased in ARSA than in ASSA at all treatments. This suggests that adhesion-related genes (*clfA*, *eno*, and *fnbA*) and efflux-related genes (*mdeA*, *norB*, and *norC*) can contribute to enhanced antibiotic resistance and bacterial pathogenicity (Huang et al. 2004). Therefore, the results emphasize the importance of controlling the ingested antibiotic-resistant pathogens in the intestinal tract. Although bacteriophages can be a promising approach to control bacterial infection, genome-based study is needed to understand the ecological interaction between bacteriophages and host bacteria in the complex gut environment (Abedon et al. 2011; Ventura et al. 2011).

In conclusion, this study highlights the potential application of bacteriophage in combination with probiotic for controlling ingested ARSA in the simulated intestinal conditions. The most significant findings in this study were that (1) high numbers of *S. aureus* exposed to the simulated gastric juices with dynamic pH and different nutrient base could reach the intestinal tract and show high sliding
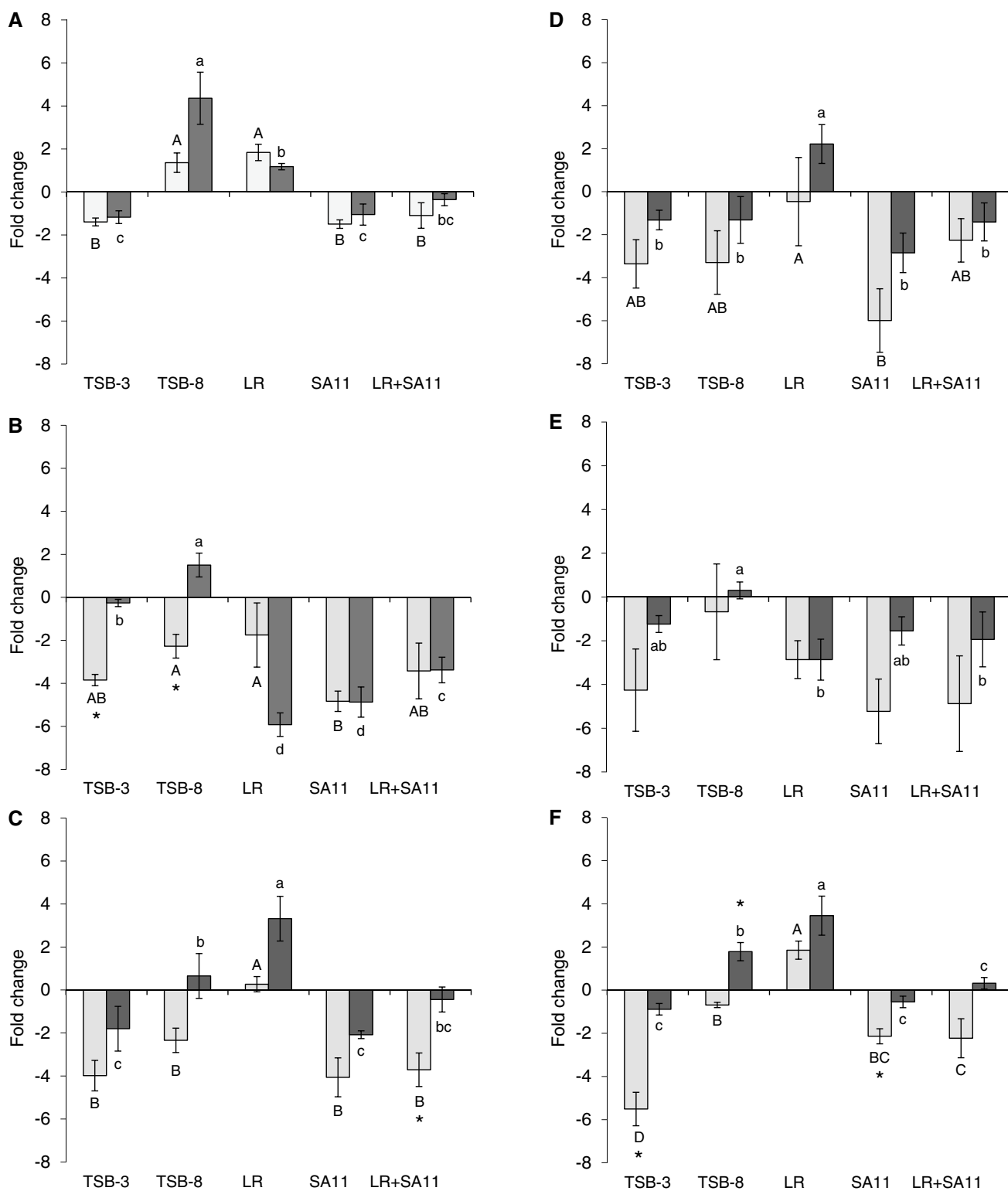
Fig. 5 Relative expression of adhesion-related (*clfA*, A; *eno*, B; and *fnbA*, C) and efflux-related (*mdeA*, D; *norB*, E; and *norC*, F) genes in *Staphylococcus aureus* exposed to the simulated gastric juice containing 0.3 % pepsin in TSB of pH 3.0 (TSB-3) and intestinal juices containing 0.03 % bile salt in TSB of pH 8.0 (TSB-8) with *L. rhamnosus* (LR), bacteriophage SA11 (SA11), and *L. rhamnosus* and SA11 (LR + SA11). Means with *different letters* (A–D) *on the bars* within antibiotic-sensitive *S. aureus* (ASSA; *grey square*) and *different letters* (**a–d**) *on the bars* within antibiotic-resistant *S. aureus* (ARSA; *filled square*) are significantly different at *P* < 0.05. *Asterisk* (*) indicates the significance between ASSA (*grey square*) and ARSA (*filled square*) at *P* < 0.05

motility responsible for bacterial attachment to the epithelial cells; (2) the bacteriophage SA11 combined with probiotic *L. rhamnosus* effectively inhibited the growth of ASSA and ARSA under the intestinal condition, which can be an alternative way to prevent foodborne bacterial infection; (3) the enhanced antibiotic resistance and virulence potential were attributed to the up-regulation of adhesion-related and efflux-related genes. These results might provide useful information to control ingested foodborne pathogens and improve intestinal microflora. Further study is needed to characterize the relationship among bacteriophages, food components, indigenous microflora, and epithelial cells in the complex GIT.

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