The inhibitory effects of *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Lactobacillus paracasei* isolated from yoghurt on the growth and enterotoxin A gene expression of *S. aureus*

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Abstract:

BACKGROUND: Staphylococcal foodborne intoxication is the most common cause of foodborne illnesses by *Staphylococcus aureus* strains and most are caused by the enterotoxins of *S. aureus*. Staphylococcal enterotoxin A (SEA) is most frequently responsible for staphylococcal food poisoning outbreaks. From a food safety and human health point of view, lactic acid bacteria (LAB) may provide a promising strategy in the fight against *S. aureus*. **OBJECTIVES:** Increasing product shelf life, and enhancing the safety of food and human health using natural microflora are the aims of this study. **METHODS:** In this study we evaluate the inhibitory effects of three *Lactobacillus strains* isolated from yoghurt, namely *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *Lactobacillus paracasei*, on the growth and enterotoxin production of *Staphylococcus aureus* by co-incubating each strain with enterotoxigenic *S. aureus* at two temperatures: 25 and 35°C. Expression of the SEA gene of *S. aureus* was assessed by real-time PCR. **RESULTS:** All the strains decreased the bacterial count at both temperatures compared to the control. This effect was greater at 25 °C than at 35°C. The production of SEA, SEC and SEE was inhibited by all the isolates tested. Furthermore, expression of the sea gene was significantly suppressed in *S. aureus* co-cultured with the lactobacillus isolates and the greatest impact was on *Lactobacillus acidophilus* at 35 °C. **CONCLUSIONS:** This research highlights the potential of lactic acid bacteria isolated from traditional foods for use as natural preservatives in foodstuffs and suggests a new approach for biocontrol of *Staphylococcus aureus*.

Introduction

Staphylococcal foodborne intoxication is the most common cause of foodborne illnesses reported in many countries and results from the consumption of foods containing sufficient amounts of one (or more) preformed enterotoxins. Symptoms of this intoxication have a rapid onset (2-8 h) and include nausea, violent vomiting and abdominal cramps with or without diarrhea. The disease is usually self-limiting and typically resolves within 24-48 h after onset. Occasionally it can be severe enough to warrant hospitalization, particularly when infants, the elderly or debilitated individuals are affected (Argudin et al., 2010; Marta et al., 2011; Schelin et al., 2011; Ventola et al., 2012; Wallin-Carlquist et al., 2010). Among food commodities that are manipulated during processing, cheeses and dry sausages are the most frequent types of food involved in *S. aureus* foodborne intoxication (Cretent et al., 2011; Dal Bello et al., 2012; Marta et al., 2011; Moslehishad et al., 2013; Rode et al., 2010; Rosengren et al., 2010). The major antigen types of staphylococcal enterotoxin are Staphylococcal enterotoxin A (SEA) to SEJ; however, additional staphylococcal enterotoxins have recently been identified (Jakobsen et al., 2011). SEs are synthesized throughout logarithmic staphylococcal growth and also during the transition from exponential to stationary phase. They are active in high nanogram to low microgram quantities, and are resistant to proteolytic enzymes and conditions (heat treatment, low pH) that easily destroy the bacteria that produce them, hence they retain their activity in the digestive tract after ingestion (Argudin et al., 2010; Drezelle et al., 2009; Larkin et al., 2009). Among the Staphylococcal enterotoxins, enterotoxin A (SEA) is that most frequently responsible for staphylococcal food poisoning outbreaks. Enterotoxigenic *S. aureus* strains have different SE gene profiles and may simultaneously harbor several genes. The expression of these virulence factors in *S. aureus* is tightly controlled and regulated by a complex network of regulatory systems. Genes encoding SE exist on various genetic supports, most of which are mobile genetic elements including phages (sea, see, sep), plasmids (sed, sej, ser, ses, set) (Adams and Moss, 2000; Cha et al., 2006; Jakobsen et al., 2011; Le Marc and Valic, 2009; Moslehishad et al., 2013; Schelin et al., 2011). Among them, the sea gene, carried in the bacterial genome by a polymorphic family of temperate bacteriophages, comprises 771 base pairs and encodes an enterotoxin A precursor of 257 amino acid residues which is expressed from the mid-exponential phase of growth (Derzelle et al., 2009; Horsmon et al., 2006; Piper et al., 2012).

Currently, increasing foodstuff shelf life as well as enhancing food safety and human health using natural microflora are dominant issues in public health. Lactic acid bacteria (LAB) have considerable potential for these purposes due to their ability to produce antimicrobial agents including organic acids, bacteriocins, hydrogen peroxide and their capacity for pH reduction. LAB are widely distributed in nature and are typically involved in a large number of spontaneous food fermentations, among which LAB isolated from dairy products have recently received much attention as potential food preservatives due to their antagonistic activity against many food borne pathogens. LAB antagonism to the growth of *S. aureus* is well documented (Chapman et al.,

2011; Charlier et al., 2009). However, the mechanism of this inhibition, and especially the inhibitory effect of LAB on *S. aureus* virulence factor expression including SE production, has not been comprehensively studied. Molecular approaches including transcriptomic and proteomic techniques are promising tools for increasing our understanding of the mechanisms involved in the growth inhibition of *S. aureus* and/or the modulation of the expression of its virulence factors by other 'safe' microorganisms, including LAB (Carey et al., 2008; Charlier et al., 2009; Laughton et al., 2006; Rode et al., 2010). The goal of this study is to evaluate the inhibitory activity of three *Lactobacillus strains*, *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *Lactobacillus paracasei*, all isolated from a traditional yoghurt, on growth and enterotoxin A production by *S. aureus* in mixed cultures.

Materials and Methods

Bacterial strains: *Staphylococcus aureus* (ATCC 29213, enterotoxin A producer) obtained from the culture collection of the Pasteur Institute, Tehran, Iran was used in this study. Three lactobacillus, *lactobacillus acidophilus*, *lactobacillus fermentum* and *lactobacillus paracasei* isolated from traditional yoghurt, were selected for antibacterial evaluation. These isolates were identified by 16s ribosomal RNA gene sequencing according with Kandler et al. (1986) and Axelsson (2004).

Preparation of inocula: *S. aureus* was sub-cultured in Trypticase Soy Broth (TSB) and incubated for 18 h at 35°C. The bacterial suspension was adjusted to an optical density (OD) of 0.1 at 600 nm using a Spectronic 20 spectrophotometer (Milton Roy Company, Houston, USA). This adjustment gave a cell concentration of 107cfu ml^{-1} as determined from previously prepared standard curve data. The number of cells in suspension was established by duplicate platings of 10-fold serial dilutions on BHI agar and counting the colonies after 24 h incubation at 35°C. *Lactobacillus cultures* were prepared by sub-culturing in MRS (de Man, Rogosa and Sharpe) broth incubated for 24 h at 35 °C and similarly titred on MRS agar.

Co-culture of *S. aureus* and lactobacillus isolates: Preparation of co-cultures of *Lactobacillus strains* and *S. aureus* was performed as described by Laughton et al. (2006) with some modifications. Each lactobacillus isolate (107cfu ml^{-1}) was grown in 10 ml TSB followed by inoculating *S. aureus* (105cfu ml^{-1}) into the medium. TSB medium inoculated individually with *S. aureus* or lactobacillus was used as controls. Cultures were incubated at 25 and 35°C for 0, 24, 48 and 72 h. Bacterial titres were determined by surface plate counting of aliquots of tenfold serial dilutions spread on MRS agar and Baird Parker agar plates for *Lactobacillus strains* and *S. aureus*, respectively.

Detection of staphylococcal enterotoxins: Detection and identification of enterotoxin types was performed using the RIDASCREEN SET (R-Biopharm GmbH, Darmstadt, Germany) visual immunoassay kit, following the instructions of the manufacturer. The kit utilizes monovalent capture antibodies against SEs types A to E.

RNA extraction and cDNA synthesis: Lactobacillus isolates were co-cultured in TSB with *S. aureus* ATCC 29213 at both 35°C and 25°C for 24 h. The effects of the *lactobacillus strains* on the expression of

the sea gene were evaluated by real-time PCR. Briefly, bacterial cultures were centrifuged in polypropylene tubes at 12.000 ×g for 5 min at 4°C. The supernatant was removed and total RNA from bacterial cells was extracted using the Tripure Isolation Reagent (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. RNA quality was monitored by measuring the absorbance at 260 and 280 nm (A260/280 ratio) using a NanoDrop spectrophotometer (Thermo Scientific, Nanodrop, Wilmington, USA). Synthesis of cDNA from 1-1.5 ng of RNA was conducted using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, St. Leon-Roth, Germany) with random hexamer primers according to the manufacturer's instructions. For each RNA sample a non-reverse transcribed control (NRTC) was included to detect contaminating genomic DNA. cDNA synthesis was performed in a DNA Engine ABI thermocycler 2720 (Applied Biosystems, Foster City, CA, USA.) using the following cycling conditions: 65ºC for 5 min, 42 ºC for 60 min and followed by 70 ºC for 5 min. Briefly, 1-1.5 ng of RNA was reverse transcribed with 1 µl random hexamer primers, 10 µM of each dNTP (2 µl), 4µl 5x first strand buffer, 1 µl Ribolock (RNase inhibitor 20 U/µl) and 1 µl RevertAidTM M-MuLV reverse transcriptase (200 U/µl).

Real-time PCR: Real-time PCR was performed on cDNA (1 µl) template in 20 µl final volume, containing 10 µl of Power SYBR Green© II PCR master mix (Thermo Scientific, Southampton, UK), 0.5 µl of each primer and 8 µl nuclease-free water. Forward and reverse sea primers were 5′-TTGGAAACGGTTAAAACGAA-3′ and 5'-GAACCTTCCCATCAAAAACA-3', respectively; forward and reverse primers for the 16s rRNA reference gene (Lee et al. 2007) were 5′-CCGCCTGGGGAGTACG-3′ and 5′-AAGGGTTGCGCTCGTTGC-3′, respectively. Real-time PCR conditions were as follows: initial denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 10 sec, primer annealing at 55 °C for 30 sec and extension at 72 °C for 30 sec, followed by melting curve analysis at 65-95 °C (temperature transition rate of 0.1 °C/sec) based on continuous fluorescence reading. Fluorescence data were collected at the end of each cycle on a Quantitative PCR system (Rotorgene-6000, Corbett, Sydney, Australia). The relative expression of the sea gene was calculated versus the calibration sample and the endogenous control (16s rRNA) to normalize the sample input amount, and the levels of sea expression of treated and untreated samples were compared. All determinations were done in triplicate.

Statistical analysis: Experimental data were analyzed using the SPSS 17.0 statistical software. Data were expressed as the mean ± SD. Statistical differences were examined using independent student's t-Test.

Table 1. Comparison of *S. aureus* enterotoxins in control cultures and mixed cultures with three lactobacillus isolates incubated at 25 and 35°C.

Incubation	control			*L. fermentum*			*L. acidophilus*			*L. paracasei*		
Temperature	SEA	SEC	SEE	SEA	SEC	SEE	SEA	SEC	SEE	SEA	SEC	SEE
25°C	+	+	+	-	-	-	-	-	-	-	-	-
35°C	+	+	+	-	-	-	-	-	-	-	-	-

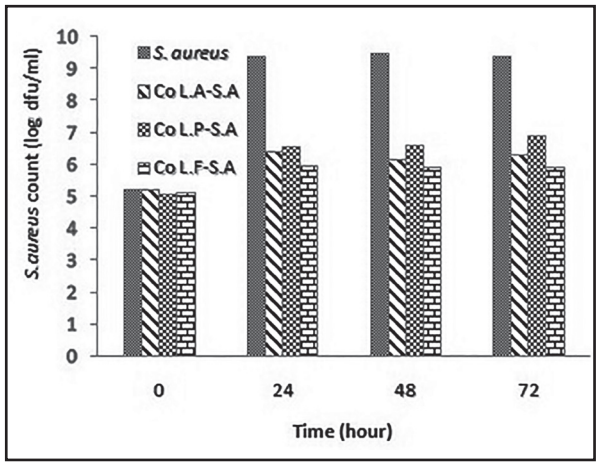

Figure 1. Comparison of logarithmic growth of *S. aureus* in co-culture with lactobacillus isolates at 25°C.

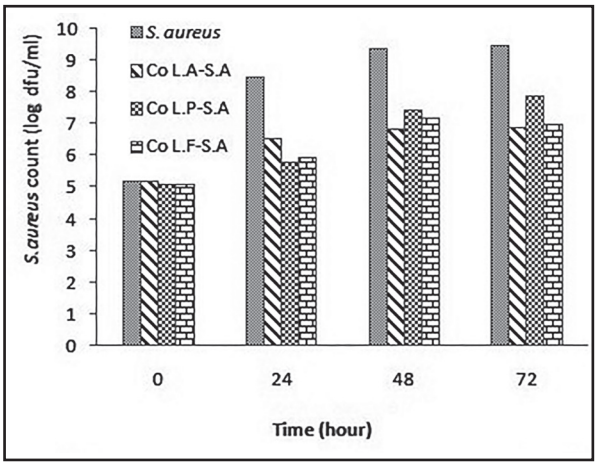

Figure 2. Comparison of logarithmic growth of *S. aureus* in co-culture with lactobacillus isolates at 35°C.

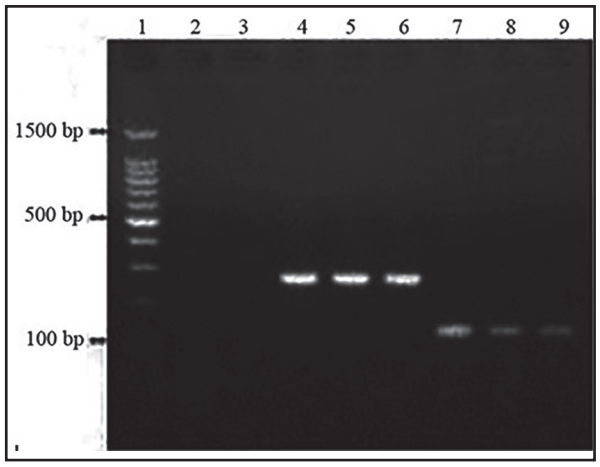

Figure 3. Agarose gel electrophoresis of the PCR products of the sea and 16s rRNA genes from cDNA templates prepared from mixed cultures of *S. aureus*. 1 Marker; 2 NTC (No Template Control); 3 NRT (No Reverse Transcriptase); 7, 8, 9 sea gene (mixed cultures: *Lb. fermentum*, *Lb. acidophilus* and *Lb. paracasei*, respectively) 120 bp; 4, 5, 6 16S rRNA gene (mixed cultures: *Lb. fermentum*, *Lb. acidophilus* and *Lb. paracasei*, respectively) 240 bp.

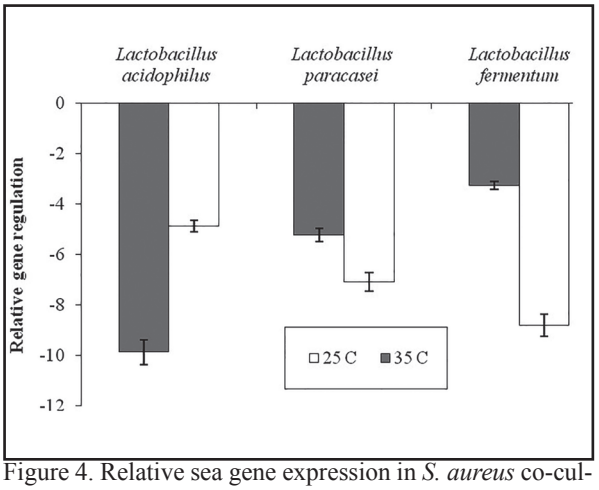

Figure 4. Relative sea gene expression in *S. aureus* co-cultured with lactobacillus isolates at 25°C and 35°C.

A p value less than 0.05 was considered to be statistically significant.

Results

Effect of lactobacillus strain on *S. aureus* growth: The effect of LAB strains on *S. aureus* counts at 25 °C is shown in Fig. 1. In the absence of LAB inoculation *S. aureus* grew during the 24 h incubation period to 9.4 log10 $CFUml^{-1}$ and bacterial counts remained unchanged over the following 48 h. Co-culture with LAB strains however had a significantly inhibitory effect on *S. aureus* growth since the cell densities of *S. aureus* co-cultured with *L. fermentum*, *L. paracasei* and *L. acidophilus* rose to only 6.3, 6.22 and 6.36 log after 24 h, respectively. No significant changes were found in *S. aureus* counts during the following 48 h incubation period. The results of *S. aureus* growth at 35 °C in the presence of LAB strains are shown in Fig. 2. LAB strains reduced the cell densities of *S. aureus* by 2-3 log compared to the control at all evaluation times. There were statistically significant differences between the results at 25 and 35 °C; the inhibition

being greater at 25 °C compared to 35 °C.

Enterotoxins assay: The pattern of toxin production by *S. aureus* in the presence of LAB strains is shown in Table 1. The production of enterotoxins (A, E, C) in the co-culture and control media was confirmed using a RIDASCREEN kit. All the LAB strains inhibited the production of SEA, SEC and SEE at 25 °C as well as at 35 °C.

Gene expression assay: The primers specific for the sea and 16s rRNA genes yield PCR products of 120 and 240 bp, respectively (Fig. 3). Real-time PCR was performed to assess sea gene expression in bacterial cultures relative to the expression of the 16s rRNA gene (endogenous control). No background signal was seen for controls lacking template or reverse transcriptase. The results of sea gene expression of *S. aureus* cultured in the presence of LAB strains at two different temperatures are shown in Fig. 4. Co-culture of *S. aureus* in the presence of each of the *Lactobacillus strains* showed reduced levels of the sea gene transcript compared to the control. However, the level of this reduction was strain and temperature dependent. At 25 °C the transcript level of sea in *S. aureus* co-cultured with *L. fermentum*, *L. paracasei* and *L. aciduphilus* was decreased 8.79, 7.08 and 4.78 fold, respectively, compared to the control, whereas at 35°C, these reductions were, 3.26, 5.21 and 9.86 fold respectively. Moreover, the greatest sea down-regulation was observed following co-incubation of *S. aureus* with *L. fermentum* at 25 °C while *L. acidophillus* showed more inhibition at 35 °C.

Discussion

From the food safety and human health points of view, inhibition of the growth and/or toxin production of foodborne pathogens including *S. aureus* by LAB are promising methods. LAB isolated from traditional foods are probably the most suitable candidates for improving the microbiological safety of foods since they are well adapted to the conditions of fermented products and should therefore be more competitive than LAB isolated from non-fermented sources. Possible mechanisms responsible for the antagonistic effects of LAB on *S. aureus* include competition for nutrients and the production of bacteriocins, hydrogen peroxide and fatty acids.

The inhibitory potential of LAB on *S. aureus* growth has been described in a number of studies (Alomar et al., 2008; Charlier et al., 2009; Cretent et al., 2011; Delbes et al., 2006; Kazemi Darsanaki et al., 2012; Otero et al., 2006; Rode et al., 2010; Rosengren et al., 2010). In the current work the effects of three *lactobacillus strains* isolated from yoghurt (*L. acidophilus*, *L. paracasei* and *L. fermentum*) on the growth of *S. aureus* were studied under co-culture conditions. The bacterial counts of *S. aureus* in co-cultures were found to be significantly lower than that in control medium, and growth inhibition at 25 °C was seen to be greater than that at 35 °C. It is well documented that the degree of growth inhibition varies depending on growth medium, the temperature and the strain tested (Chapman et al., 2011; Cotter et al., 2013; Lee and Salminen, 2009; Varalakshmi et al., 2014). Our experimental growth results are in accordance with Troller and Frazier (1963) who reported that the maximum inhibition of growth of *S. aureus* in association with other organisms occurred at temperatures of 20 to 25 °C. Pereira et al. (2009) reported similar findings regarding the inhibition of *S. aureus* by psychrophilic

saprophytes. Previous reports also suggest that the growth of *S. aureus* is inhibited to a greater degree at temperatures lower than 30 °C when cultured in the presence of other microorganisms (Alomar et al., 2008; Dos Santos et al., 2016; Haines and Harmon, 1973; Troller and Frazier, 1963).

Among *S. aureus* virulence factors, enterotoxins play a particularly important role and are the principal threat in foodstuffs. In our study, the results of enterotoxin detection show that the production of SEA, SEC and SEE is suppressed in the presence of the lactobacillus isolates studied. This inhibitory effect has also been reported by other researchers (Haines and Harmon, 1973; Troller and Frazier, 1963). Despite the importance of enterotoxins, regulation of their expression and that of enterotoxin-like proteins is still poorly documented. Moreover, the effect of bacteria-bacteria interactions on enterotoxin gene expression in *S. aureus* has not been well studied.

Molecular approaches including transcriptomics are promising methods for increasing our knowledge of the mechanisms involved in the inhibition of the production of bacterial virulence factors via microbial interactions (Garzoni et al., 2007; Rode et al., 2010). Indeed, reverse transcriptase PCR has been used previously to study *S. aureus* SEs expression (Hor et al., 2014; Wallin-Carlquist et al., 2010; Young-Duck et al., 2007). In the present work the effects of lactobacillus isolates on sea expression levels in *S. aureus* was investigated employing real-time PCR because of its increased sensitivity and specificity compared to conventional methods (Garzoni et al., 2007; Horsmon et al., 2006; Ruijter, 2009). We have shown that lactobacillus isolates reduce sea expression in *S. aureus*. Specifically, our data reveal that co-culture with *L. fermentum* results in the greatest down regulation of sea gene expression at 25 °C, whilst at 35 °C this is achieved by *L. acidophilus*. To date, very few gene expression studies have been carried out to evaluate the impact of probiotic bacteria on the gene expression of SEs or other exotoxins (Cotter et al., 2013; Even et al. 2009). Recently, Laughton et al. (2006) reported that *L. ruteri* produces a low molecular weight soluble compound which is able to interfere with the expression of an exotoxin gene in *S. aureus*. It was indicated that the impact of *L. lactis* on enterotoxin expression was enterotoxin type dependent; *L. lactis* strongly decreased the expression of sec and sel, while slightly favoring the expression of sea (Even et al., 2009).

In conclusion, the results of this study reveal that the lactobacillus isolates studied reduced both *S. aureus* growth and its production of enterotoxin. Real time PCR analysis showed down-regulation of sea which was both temperature and strain dependent. These results indicate the potential for application of these isolates as natural agents for the prevention of bacterial growth/toxin production with a view to improving the quality and safety of susceptible foods.

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References

Adams, M.R., Moss, M.O. (2000) Staphylococcal enterotoxins. In: Food Microbiology. Adams, M.R. (ed.).(2nd ed.) Royal Society of Chemistry, Cambridge, UK. p. 54-64 and 253-259.

Alomar, J., Loubiere, P., Delbes, C., Nouaille, S., Montel, M.C. (2008) Effect of *Lactococcus garvieae*, *Lactococcus lactis* & *Enterococcus faecalis* on the behavior of *Staphylococcus aureus* in microfiltered milk. Food Microbiol. 25: 502 -508.

Argudin, M.A., Mendoza, M.C., Maria Rosario Rodicio, M.R. (2010) Food Poisoning and *Staphylococcus aureus* Enterotoxins. Toxins. 2: 1751-1773.

Axelsson, L. (2004) Lactic acid bacteria: classification and physiology. In: Lactic Acid Bacteria; Microbiological and Functional Aspects. Salminen, S., Wright, A.V., Ouwehand, A. (eds.). Marcel Dekker, New York, USA. p. 1-67.

Carey, C.M., Kostrzynska, M., Ojha, S., Thompson, S. (2008) The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic *Escherichia coli* O157:H7. J Microbiol Methods. 73: 125-132.

Cha, J.O., Lee, J.K., Jung, Y.H., Yoo, J.I., Park, Y.K., Kim, B.S., Lee, Y.S. (2006) Molecular analysis of *Staphylococcus aureus* isolates associated with staphylococcal food poisoning in South Korea. J Appl Microbiol. 101: 864-871.

Chapman, C.M.C., Gibson, G.R., Rowland, I. (2011) Health benefits of probiotics: are mixtures more effective than single strains? Eur J Nutr. 50: 1-17.

Charlier, C., Cretenet, M., Even, S., Loir, Y.L. (2009) Interactions between *Staphylococcus aureus* and lactic acid bacteria: an old story with new perspectives. Int J Food Microbiol. 131: 30-39.

Cotter, P.D., Ross, R.P., Hill, C. (2013) Bacteriocins—a viable alternative to antibiotics? Nat Rev Microbiol. 11: 95-105.

Cretenet, M., Nouaille, S., Thouin, J., Rault, L., Stenz, L., François, P., Hennekinne, J.A., Piot, M., Maillard, M.B., Fauquant, J., Loubière, P., Le Loir, Y., Even, S. (2011) *Staphylococcus aureus* virulence and metabolism are dramatically affected by *Lactococcus lactis* in cheese matrix. Environ Microbiol Reports. 3: 340-351.

Dal Bello, L., Cocolin, G., Zeppa, D., Field, P.D., Cotter, C. Hill, C. (2012) Technological characterization of bacteriocin producing *Lactococcus lactis* strains employed to control Listeria monocytogenes in Cottage cheese. Int J Food Microbiol. 153: 58-65.

Delbes, C., Alomar, J., Chougui, N., Martin, J.F., Montel, M.C. (2006) *Staphylococcus aureus* growth and enterotoxin production during the manufacture of uncooked, semihard cheese from cows' raw milk. J Food Prot. 69: 2161-2167.

Derzelle, S., Dilasser, F., Duquenne, M., Deperrois, V. (2009) Differential temporal expression of the staphylococcal enterotoxins genes during cell growth. Food Microbiol. 26: 896-904.

Dos Santos. T.F., Melo, T.A, Santos D.S., Rezende, R.P., Dias, J.C.T., Romano, C.C. (2016) Efficacy of oral administration of lactic acid bacteria isolated from cocoa in a fermented milk preparation: reduction of colitis in an experimental rat model. Genet Mol Res. 5.

Even, S., Charlier, C., Nouaille, S., Ben Zakour, N.L., Cretenet, M., Cousin, F.J. Gautier, M., Cocaiqn-Bousquet, M., Loubiere, P., Loir, Y.L. (2009) *Staphylococcus aureus* virulence expression is impaired by *Lactococcus lactis* in mixed cultures. Appl Environ Microbiol. 75: 4459-72.

Garzoni, C., Francois, P., Huyghe, A., Couzinet, S., Tapparel, C., Charbonnier, Y., Renzoni, A., Lucchini, S., Lew, D.P., Vaudaux, P., Kelley, W.L., Schrenzel, J. (2007) A global view of *Staphylococcus aureus* whole genome expression upon internalization in human epithelial cells. BMC Genomics. 8: 171.

Haines, W.C., Harmon, L.G. (1973) Effect of variations in conditions of incubation upon inhibition of *Staphylococcus aureus* by Pediococcus cerevisiae and *Streptococcus lactis*. Appl Microbiol. 25: 169-172.

Hor, Y.Y., Liong, M.T. (2014) Use of extracellular extracts of lactic acid bacteria and bifidobacteria for the inhibition of dermatological pathogen *Staphylococcus aureus*. Dermatol Sin. 32: 141-7.

Horsmon, J.R., Cao, C.J., Khan, A.S., Gostomski, M.V., Valdes, J.J., O`Connell, K.P. (2006) Real-time fluorogenic PCR assays for the detection of entA, the gene encoding staphylococcal entrotoxin A. Biotechnol Lett. 28: 823- 829.

Jakobsen, R.A., Heggebo, R., Sunde, E.B., Skjervheim, M. (2011) *Staphylococcus aureus* and Listeria monocytogenes in Norwegian raw milk cheese production. Food Microbiol. 28: 492-496.

Kandler, O., Weiss, N. (1986) Regular, nonsporing Gram-positive rods. In: Bergey's manual of systematic bacteriology.Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G. (eds.). Williams and Wilkins. Baltimore, USA. p. 1209-1235.

Kazemi Darsanaki, R., LalehRokhi, M., Azizollahi, M., Issazadeh, K.H. (2012) Antimicrobial Activities of *Lactobacillus strains* Isolated from Fresh Vegetables. Middle-East J Sci Res. 11: 1216-1219.

Larkin, E.A., Carman, R.J., Krakauer, T., Stiles, B.G. (2009) *Staphylococcus aureus*: the toxic presence of a pathogen extraordinaire. Cur Med Chem. 16: 4003-4019.

Laughton, J.M., Devillard, E., Heinrichs, D.E., Reid, G. McCormick J.K. (2006) Inhibition of expression of a staphylococcal superantigen-like protein by a soluble factor from *Lactobacillus reuteri*. Microbiol. 152: 1155-1167.

Lee, Y.K., Salminen, S. (2009) Handbook of probiotics and prebiotics. (2nd ed.) John Wiley and Sons, Inc., Hoboken, New Jersey, USA.

Le Marc, Y., Valic, L., Medvedova, A. (2009) Modelling the effect of the starter culture on the growth of *Staphylococcus aureus* in milk. Int J Microbiol. 129: 306- 311.

Márta, D., Wallin-Carlquist, N., Schelin, J., Borch, E., Radstrom, P. (2011) Extended staphylococcal enterotoxin D expression in ham products. Food Microbiol. 28: 617-620.

Moslehishad, M., Ehsani, M.R., Salami, M. (2013) The comparative assessment of ACE-inhibitory and antioxidant activities of peptide fractions obtained from fermented camel and bovine milk by *Lactobacillus rhamnosus* PTCC 1637. Int Dairy J. 29: 82-87.

Otero, M.C., Nader-Macias, M.E. (2006) Inhibition of *Staphylococcus aureus* by H2O2-producing *Lactobacillus gasseri* isolated from the vaginal tract of cattle. Anim Reprod Sci. 96: 35-46.

Pereira, V., Lopes, C., Castro, A., Silva, J., Gibbs, P. Teixeira, P. (2009) Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. Food Microbiol. 26: 278-282.

Piper, C., Casey, P.G., Hill, C., Cotter, P.D., Ross, R.P. (2012) The lantibiotic lacticin 3147 prevents systemic spread of *Staphylococcus aureus* in a murine infection model. Int J Microbiol. Article ID 806230, 6 pages.

Rode, T.M., Moretro, T., Langsrud, S., Langsrud, O., Vogt, G., Holck, A. (2010) Respons-

es of *Staphylococcus aureus* exposed to HCl and organic acid stress. Can J Microbiol. 56: 777-792.

Rosengren, A., Fabricius, A., Guss, B., Sylven, S., Lindqvist, R. (2010) Occurrence of food-borne pathogens and characterization of *Staphylococcus aureus* in cheese produced on farmdairies. Int J Food Microbiol. 144: 263-269.

Ruijter, J.M. (2009) Amplification efficiency: linking baseline & bias in the analysis of quantitative PCR data. Nucleic Acids Res. 37: 202-207.

Schelin, J., Wallin-Carlquist, N., Thorup Cohn, M., Lindqvist, R., Barker, G.C. (2011) The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. Virulence 2: 580-592.

Troller, J., Frazier, C. (1963) Repression of *Staphylococcus aureus* by food bacteria. I Effect of environmental factors on inhibition. Appl Microbiol. 11: 11-14.

Varalakshmi, S., Balasubramanyam, B.V., Surendranath, B., Bagath, M., Rajendran, D. (2014) Use of novel lactic acid bacterial strains with antagonistic activity for the preparation of safe indigenous fermented dairy foods (Dahi and Raita). J Food Safety. 34: 26-33.

Ventola, H., Lehtoranta, L., Madetoja, M. (2012) Effects of the viability of *Lactobacillus rhamnosus* GG on rotavirus infection in neonatal rats. World J Gastroenterol. 18: 5925-5931.

Wallin-Carlquist, N., Cao1, R., Marta1, D., Sant'Ana da Silva1, A., Schelin, J., Radstrom, P. (2010) Acetic acid increases the phage-encoded enterotoxin A expression in *Staphylococcus aureus*. BMC Microbiol. 10: 147.

Young-Duck, L., Moon, B.Y., Park, J.H., Chang, H.I., Im, W. (2007) Expression of entrotoxin genes in *Staphylococcus aureus* isolates based on mRNA analysis. J Microbiol Biotechnol. 17: 461-467.

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بررسی اثر مهاری ایزوله های لاکتوباسیلوس فرمنتوم، لاکتوباسیلوس اسیدوفیلوس و لاکتوباسیلوس پارا کازئی جدا شده از ماست بر روی رشد و بیان ژنی انتروتوکسین A باکتری استافیلو کوکوس اورئوس

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چکیده

زمینه مطالعه: مسمومیت غذایی استافیلوکوکی رایج ترین مسمومیت غذایی توسط سویه‌های استافیلو کوکوس اورئوس می‌باشد و عمدتاً توسط انتروتوکسین‌های استافیلو کوکوس اورئوس ایجاد می‌شود. بیشترین توکسین درگیر درمسمومیت‌های غذایی استافیلوکوکی، انتروتوکسین A (SEA) است. از نقطه نظر ایمنی غذایی و سلامت انسانی، باکتری های اسید لاکتیک یک استراتژی امیدوار کننده در مبارزه با استافیلو کوکوس اورئوس را ارائه می دهند. **هدف:** افزایش ماندگاری، ایمنی مواد غذایی و ارتقای سلامتی انسان با استفاده از میکرو فلور طبیعی از اهداف این مطالعه محسوب می شوند. **روش کار:** در این تحقیق اثر جدایه های لاکتوباسیلوس جدا شده از ماست بنام لاکتوباسیلوس فرمنتوم، لاکتوباسیلوس اسیدوفیلوس و لاکتوباسیلوس پارا کازئی بر رشد و تولید انتروتوکسین‌های باکتری استافیلو کوکوس اورئوس با گرمخانه گذاری همزمان در دو دمای ° ۲۵ و ۳۵ ارزیابی گردید و سپس بیان ژن انتروتوکسین A باکتری استافیلو کوکوس اورئوس، به روش Real time PCR مورد بررسی قرار گرفت. **نتایج:** تمامی جدایه های لاکتوباسیل در مقایسه با کنترل رشد باکتری استاف را در دو دمای گرمخانه گذاری کاهش دادند و این کاهش اثر در دمای ° ۲۵ بیشتر از ° ۳۵ بوده است. تولید انتروتوکسین های A، C و E توسط جدایه ها مهار گردید. علاوه بر این، بیان ژن sea بطور معنی‌داری در کشت همزمان استاف با جدایه های لاکتوباسی ل کاهش یافت و بیشترین اثر کاهشی مربوط به لاکتوباسیلوس اسیدوفیلوس در دمای ° ۳۵ بوده است. **نتیجه‌گیری‌نهایی:** این پژوهش پتانسیل کاربرد باکتری های اسید لاکتیک جدا شده از فراورده های غذایی سنتی به عنوان نگه دارنده های طبیعی در مواد غذایی را نشان می دهد و یک روش جدید برای کنترل زیستی استافیلو کوکوس اورئوس را پیشنهاد می دهد.

واژه های کلیدی: بیان ژن، لاکتوباسیلوس اسیدوفیلوس، لاکتوباسیلوس فرمنتوم، لاکتوباسیلوس پارا کازئی ، استافیلو کوکوس اورئوس

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