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The Inhibitory Effects of 2 Commercial Probiotic Strains on the Growth of *Staphylococcus aureus* and Gene Expression of Enterotoxin A

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Abstract

Background: Food-borne intoxications are current problems in human society and most of them are caused by the enterotoxins of *Staphylococcus aureus*. Staphylococcal enterotoxin A (SEA) is the 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 to combat the pathogenic bacteria, particularly *S. aureus*.

Objective: The objective of this study was to evaluate the inhibitory activity of two commercial lactobacillus strains on growth and enterotoxin A production by *S. aureus*. Moreover, the inhibitory effect of these strains on gene expression of enterotoxin type A was assessed using real-time Polymerase chain reaction (PCR).

Materials and Methods: In this study the inhibitory effect of two commercial probiotic strains, *Lactobacillus acidophilus* (LA5) and *Lactobacillus casei* 01 on the growth and enterotoxin production of *S. aureus* was evaluated at 25 and 35°C. The gene expression of SEA of *S. aureus* was also evaluated by real time (RT) PCR technique.

Results: The lactobacillus strains decreased the bacterial count at both temperatures compared with the control group. This reduced effect was greater at 25°C (3 log/CFU) than 35°C (2 log/CFU). The production of SEA, SEC and SEE was inhibited by the lactobacillus strains. Furthermore, the gene expression of SEA was significantly suppressed in *S. aureus* co cultured with studied lactobacillus strains and the greatest down-regulation of *sea* (10.31 fold) was observed in co-incubation of *S. aureus* with LC01 at 25°C.

Conclusion: This research raises important implications for the potential use of LAB as a natural preservative in foodstuffs by correct microbial ecology of the environment and a new approach for biocontrol of *S. aureus*.

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Background

Food-borne intoxications are current problems in human society and most of them are caused by the enterotoxins of *Staphylococcus aureus*.[1] Staphylococcal food poisoning (SFP) is due to the production of staphylococcal enterotoxins (SEs) by *Staphylococcus aureus* strains contaminating foodstuffs such as meat and meat products, poultry and egg products, milk and dairy products, salads, bakery products, particularly cream-filled pastries and cakes, and sandwich fillings.[2-4] The control of this disease is of social and economic importance as it represents a considerable burden in terms of loss of working days and productivity, hospital expenses, and economic losses in food industries, catering companies and restaurants.[2,5]

SEs are synthesized throughout the logarithmic phase of growth during the transition from exponential to stationary phase. They are active in high nanogram to low microgram quantities and are resistant to physical conditions (heat treatment and low pH) that easily destroy the bacteria that produce them as well as proteolytic enzymes, and hence retain their activity in the digestive tract after ingestion.[2,6] Staphylococcal enterotoxin A (SEA) is the most frequently responsible for staphylococcal food poisoning outbreaks. The expression of virulence factors in *S. aureus* is tightly controlled by a complex network of regulatory systems. Genes encoding SEs are carried on various genetic supports, most of which are mobile genetic elements including phages (*sea, see* and *sep*) and plasmids (*sed, sej, ser, ses* and *set*).[7] However, the *sea* gene, carried in the bacterial genome by a polymorphic family of temperate bacteriophages, which is composed of 771 base pairs and encodes enterotoxin A precursor of 257

amino acid residues which is expressed from the mid-exponential phase of growth.[8]

The inhibition of *S. aureus* growth and production of SE in foodstuffs is of importance in the public health, therefore, to detect the prevalence of enterotoxic strains in foods is required. From a food safety and human health point of view, lactic acid bacteria (LAB) may provide a promising strategy to combat against *S. aureus*. In recent years interactions between *S. aureus* and LAB have been examined in several ecosystems, including fermented foodstuffs as well as the nasal and vaginal environments.[3,9] However, studies of the inhibition of *S. aureus* virulence expression by LAB, including the inhibition of SE production, are quite scarce. Few studies have described the inhibition of enterotoxin production in the presence of LAB, and none have unraveled the mechanisms involved in such antagonism.[9]

Objectives

The objective of this study was to evaluate the inhibitory activity of 2 commercial *Lactobacillus* strains - *Lactobacillus acidophilus* (LA5) and *Lactobacillus casei* 01 - on growth and enterotoxin A production by *S. aureus* in mixed cultures. Moreover, the inhibitory effect of these strains on gene expression of enterotoxin type A was assessed using real time polymerase chain reaction (PCR).

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. Two probiotic strains, *L. acidophilus* (LA5) and *L. casei* 01, were obtained from the Christian Hansen company.

Preparation of Inocula

Staphylococcus aureus was sub-cultured in Trypticase Soy Broth (TSB) and incubated for 18 hours 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 10^7 CFU/mL as determined from previously prepared standard curve data. The number of cells in the suspension was enumerated by duplicate plating from 10-fold serial dilutions on BHI agar and counting the colonies after 24 hours incubation at 35°C. *Lactobacillus* cultures were prepared by sub-culturing in MRS (de Man, Rogosa and Sharpe) broth incubated for 24 hours at 35°C and similarly titred on MRS agar.

Co-culture of *Staphylococcus aureus* and *Lactobacillus* Commercial Strains

Preparation of co-cultures of *Lactobacillus* strains and *S. aureus* was done as described by Laughton et al[10] with some modifications. Each *Lactobacillus* strain (10^7 CFU/mL) was grown in 10 mL TSB followed by inoculating *S. aureus* (10^5 CFU/mL) into the medium. TSB medium inoculated with *S. aureus* or *Lactobacillus* individually were considered to be controls. Cultures were incubated at 25 and 35°C for 0, 24, 48 and 72 hours. 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

The RIDASCREEN SET kit (R-Biopharm GmbH, Darmstadt, Germany), a commercial SE visual immunoassay kit, was used to evaluate the presence of SEs. The kit utilizes monovalent capture antibodies against SEs types A to E. Detection and identification of the enterotoxin types was performed as recommended by the manufacturer.

RNA Extraction and cDNA Synthesis

Lactobacillus strains were co-cultured in TSB with *S. aureus* ATCC 29213 at both 35°C and 25°C for 24 hours. The effect of *Lactobacillus* commercial strains on gene expression of SEA was evaluated by real-time PCR (RT-PCR). Briefly, bacterial cultures were centrifuged in polypropylene tubes at 12 000 ×g for 5 minutes at 4°C. The supernatant was removed and total RNA from bacterial cells was extracted using 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 (Fermentase, 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. The cDNA synthesis was performed in a DNA Engine ABI thermocycler 2720 (Applied Biosystems, Foster City, CA, USA) using the following cycling conditions: 65°C for 5 minutes, 42°C for 60 minutes and followed by 70°C for 5 minutes. Briefly, 1-1.5 ng of RNA was reverse transcribed with 1 mL random hexamer primers, 10 µM of each dNTP (2 µL), 4 µL 5x first strand buffer, 1 µL Ribolock (RNase inhibitor 20 U/µL), and 1 µL RevertAid™ M-MuLV reverse transcriptase (200 U/µL). For each RNA sample a NRTC was included.

Real-Time Polymerase Chain Reaction

RT-PCR was used to assess SEA gene expression using *16s rRNA* as the endogenous control. The cDNA (1 µL) was used as a template for amplification in 20 µL final volume, containing 10 µL of power SYBR Green© II PCR master mix (Primer design, 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 reference *16s rRNA* gene were 5′-CCGCCTGGGGAGTACG-3′ and 5′-AAGGGTTGCGCTCGTTGC-3′, respectively.[11] RT-PCR conditions were as follows: initial denaturation at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds, primer annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds, followed by melting curve analysis at 65-95°C (temperature transition rate of 0.1°C/s) 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

All experiments were repeated three times. Bacterial growth curves were drawn using GraphPad Prism 4 software and statistical analyses of both bacterial counts after different periods (24, 48 and 72 hours) and also the gradients of the growth curves were conducted using SPSS 16 software at temperatures of 25 and 35°C. One-way analysis of variance (ANOVA) in combination with the Tukey test was used to do mean comparisons and the differences between mean values were significant at the 5% confidence level. Statistical significance between treatments and the control group was assessed by REST© (Relative expression software tool 2009). A significant difference was defined as a P value <0.05.

Results

Effects of LA5 and LC01 on *Staphylococcus aureus* Growth

The growth of *Staphylococcus aureus* in either the presence or absence of *Lactobacillus* strains LC01 and LA5 was monitored at both 25 and 35°C. Growth curves of *S. aureus* shown in Figure 1 indicate that after 24 hours of incubation until the end of the experiment the titre of *S. aureus* in the control group was significantly greater than the corresponding titres in either of the other treatments ($P<0.001$) at 25°C. In addition, the titre measured in co-culture with LA5 during the last 48 hours of the experiment was significantly lower than that of the co-culture with LC01 ($P<0.05$). The comparison of linear gradient of *S. aureus* growth at 25°C showed that the increasing trend of bacteria number (gradient at 0.052 ± 0.01) was significantly higher at control group in comparison with groups containing LC01 (gradient at 0.028 ± 0.003) ($P=0.018$) and also with groups containing LA5 (0.006 ± 0.004) ($P<0.001$); also the increasing trend was significantly higher in LC01 than LA5 ($P=0.029$).

The results presented in Figure 2 corresponding to *S. aureus* growth at 35°C show similar trends to those seen at 25°C. The titre of *S. aureus* was always greater in the control than in the LC01 and LA5 treated samples ($P<0.001$) and the LA5 treated sample showed significantly lower numbers of *S. aureus* compared with the LC01 sample during the final 48 hours of the experiment ($P<0.05$).

Enterotoxin Assay

Enterotoxin production by *S. aureus* is shown in Table 1. Whilst LC01 inhibited the production of SEA at both temperatures, LA5 showed no observable inhibitory effect.

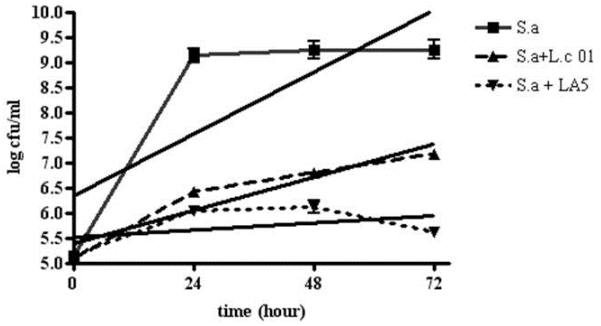

Figure 1. Logarithmic Plot of *Staphylococcus aureus* Growth in the Presence of LC01 and LA5 Bacteria vs the Control Culture at 25°C.

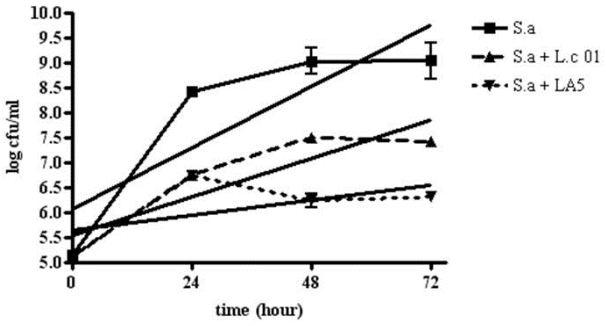

Figure 2. Logarithmic Plot of *Staphylococcus aureus* Growth in the Presence of LC01 and LA5 Bacteria vs the Control Culture at 35°C.

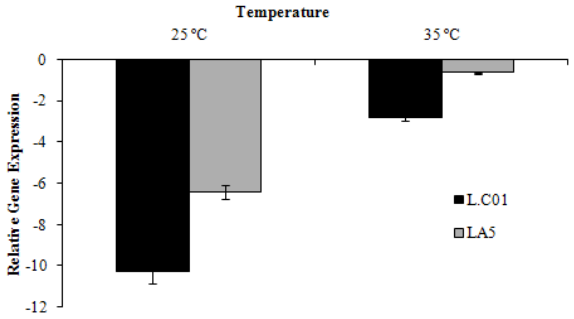

Figure 3. Relative *sea* Gene Expression of *Staphylococcus aureus* Co-cultured With Commercial Strains of *Lactobacillus* at 25 and 35°C.

Table 1. Comparison of *Staphylococcus aureus* Enterotoxin Production in the Mixed Cultures with Commercial Strains of *Lactobacillus* Incubated at 25 and 35°C After 72 Hours

Incubation Temperature	Control			LA5			*Lactobacillus casei* 01		
	SEA	SEC	SEE	SEA	SEC	SEE	SEA	SEC	SEE
25°C	+	+	+	-	-	-	-	-	-
35°C	+	-	+	-	-	-	-	-	-

Gene Expression Assay

The analysis of *sea* gene expression by *S. aureus* co-cultured in the presence of commercial *Lactobacillus* strains at 2 different temperatures is presented in Figure 3. All co-cultures showed down-regulation of the target gene compared with the control group. However, this reduction was dependent on both temperature and the presence of *Lactobacillus* strain. For example, at 25°C the transcriptional levels of *sea* in *S. aureus* co-cultured with *LA5* and *LC01* were respectively 6.42 and 10.31 fold lower than the control group, whereas at 35°C this reduction was 2.83 fold for LC01 and that for LA5 was not significant ($P > 0.05$). The greatest down-regulation of *sea* was observed in co-incubation of *S. aureus* with *LC01* at 25°C.

Discussion

Staphylococcus aureus can grow across a wide range of environmental conditions and is a frequent contaminant of food. This contamination can originate from raw materials (eg, mastitic milk), from the processing plant environment (eg, biofilms on processing surfaces) or from handlers activity during food preparation and manipulation. The frequency of *S. aureus* contamination and the effect of staphylococcal food poisoning incidences on public health justify the interest taken by the scientific community and agro-food industries in combating this problem. A great deal of attention has also recently been given to certain foods as potential vehicles for antimicrobial compounds. Such foods have become important health care sectors in most countries, and among them, dairy products containing LAB such as *Lactobacilli* are of particular relevance (12). LAB can produce antimicrobial substances with the capacity to inhibit the growth of pathogens and spoilage microorganisms. In this study the effects of two commercial *Lactobacillus* strains (*L. acidophilus* LA5 and *L. casei* 01), isolated from some fermented dairy products, and were studied on the growth, enterotoxin production and *sea* gene expression in *S. aureus* under co-culture conditions.

The inhibitory potential of LAB on *S. aureus* growth has been described in various studies.[13-15] The results of the present study indicate that at 25°C the inhibition of both *S. aureus* growth and *sea* expression is greater than that at 35°C in both *Lactobacillus* co-cultures. Sameshima et al and Gonzalez-Fandos et al indicated that *S. aureus* growth inhibition varied depending on the temperature.[16,17] Troller and Frazier reported that maximum inhibition of *S. aureus* growth, in association with other organisms, occurred at temperatures of 20 to 25°C, supporting our results.[18] Other previous reports also suggest that growth of *S. aureus* is generally inhibited to a greater degree at temperatures lower than 30°C when cultured with other organisms.[13,19]

A number of propositions have been made to explain the mechanism of inhibition of *S. aureus* growth by LAB, including the production of bacteriocins and hydrogen peroxide,[15,19] competition for nutrients, and acidification.[3] LAB are known to produce a range of antimicrobial compounds including lactic acid, acetic acid, formic acid, phenyllactic acid, caproic acid, organic acids, ethanol, hydrogen peroxide, diacetyl, bacteriocins, reuterin, reutericyclin and bactericidal proteins.[20,21] It was previously demonstrated that *L. acidophilus* LA5 secreted molecules influenced on pathogens as *E. coli* O157 secretion system.[22] Wang et al detected the down regulation of important virulence-related gene expression after *S. aureus*, *E. coli* O157 and *Shigella flexneri* were grown in medium supplemented with biologically active fractions of *L. acidophilus* La-5 CFSM compared with the same bacteria grown in the same medium without the addition of La-5 fractions. They also found that the count of *S. aureus* decreased after mixing with *L. acidophilus* LA5 and *L. acidophilus* ATCC 4356 over 1 hour.[23] Dicks and Botes[24] had reported that hydrogen peroxide produced by some strains of Lactobacilli, effectively inhibits *S. aureus*, and *L. acidophilus* isolated from humans due to production of bacteriocin and non-bacteriocin antimicrobial substances which are active (both in vitro and in vivo) tests against gram-positive and gram-negative pathogens.[24] Tomioka et al has investigated the effect of ofloxacin combined with *L. casei* against *Mycobacterium fortuitum* induced infection in mice. They found a marked delay in the incidence of spinning disease and an increase in the rate of elimination of organisms from the kidneys.[25] Among the virulence factors produced by *S. aureus*, enterotoxins are the main health threat in foodstuffs. In the present study, the effect of *Lactobacillus* strains on the level of *sea* gene expression was investigated by RT-PCR given the latter's increased sensitivity and specificity compared to the conventional method.[8,26] RT-PCR has been used previously to study *S. aureus* SE gene expression.[27] Our data show that *S. aureus* co-culture with *L. casei* 01 resulted in the greatest level of down-regulation of *sea* gene expression at 25°C compared with *L. acidophilus* (LA5). At 35°C, *L. acidophilus* (LA5) was ineffective in inhibiting SEA production by *S. aureus* whilst co-culture with *L. casei* 01 showed weak *sea* down-regulation. Molecular approaches including transcriptomic ones are promising for increasing our

knowledge of the mechanism involved in inhibition of bacterial virulence factors by microbial interactions. In the present study, we showed by transcriptional expression analysis that lactobacillus isolates reduced *sea* expression in *S. aureus*. Very few gene expression studies have been carried out to evaluate the impact of probiotic bacteria on the gene expression of SEs as well as other exotoxins.[3] Recently, Laughton et al reported that *Lactobacillus ruteri* produces a small-sized soluble compound which is able to interfere with the expression of an exotoxin gene in *S. aureus*.[10] It was indicated that the impact of *Lactococcus lactis* on enterotoxin expression was enterotoxin type dependent; *L. lactis* strongly decreased the expression of *sec* and *sel*, while slightly favored the expression of *sea*.[9] As it is observed from the results of the present study, *L. acidophilus* (LA5) had stronger inhibitory activity against the growth of *S. aureus* while *L. casei* 01 showed the greatest down regulation of *sea*. It is reported that the expression of virulence of an *S. aureus* strain may also be inhibited, even if no prevention of growth is occurred.[10]

The results of this study revealed that co-culture of *S. aureus* with 2 commercial *Lactobacillus* strains resulted in reductions in both growth (*L. acidophilus* was the most effective) and enterotoxin production (*L. casei* had the greatest effect). Furthermore RT-PCR analysis revealed down-regulation of the *sea* gene, a phenomenon which was also temperature and strain dependent. Our data therefore suggest that these isolates could be considered to be applied as natural inhibitory agents against bacterial growth and toxin production in order to maintain the quality and improve the safety of certain food products.

Authors' Contributions

MP: Designing the study, Obtaining the samples and writing the manuscript; MA: drafting of the manuscript; AJJ: Conducting the statistical analyses.

Ethical Approval

All procedures performed in this study were in accordance with the ethical standards of the national research committee.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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References

1. Stiles BG, Krakauer TK. Staphylococcal enterotoxins: a purging experience in review, Part 1. Clin Microbiol News. 2005;27(24):179-186.
2. Argudin MA, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins. 2010;2(7):1751-1773. doi:10.3390/toxins2071751.
3. Even S, Charlier C, Nouaille S, et al. *Staphylococcus aureus* virulence expression is impaired by *Lactococcus lactis* in mixed cultures. Appl Environ Microbiol. 2009;75(13):4459-4472.
4. Morandi S, Brasca M, Lodi R, Cremonesi P, Castiglioni B. Detection of classical enterotoxins and identification of enterotoxin genes in *Staphylococcus aureus* from milk and dairy products. Vet Microbiol. 2007;124(1-2):66-72.
5. Larkin EA, Carman RJ, Krakauer T, Stiles BG. *Staphylococcus aureus:* the toxic presence of a pathogen extraordinaire. Curr Med Chem. 2009;16(3):4003-4019.
6. Derzelle S, Dilasser F, Duquenne M, Deperrois V. Differential temporal expression of the staphylococcal enterotoxins genes during cell growth. Food Microbiol. 2009;26(8):896-904.
7. Akinedan O, Hassan AA, Schneider E, Usleber E. Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats' milk cheese. Int J Food Microbiol. 2008;31(2):211-216.
8. Horsmon JR, Cao CJ, Khan AS, Gostomski MV, Valdes JJ, O`connell KP. Real-time fluorogenic PCR assays for the detection of ent A, the gene encoding staphylococcal entrotoxin A. Biotechnol Lett. 2006;28(11):823-829.
9. Charlier C, Cretenet M, Even S, Loir YL. Interactions between Staphylococcus aureus and lactic acid bacteria: an old story with new perspectives. Int J Food Microbiol. 2009;131(1):30-39.
10. Laughton JM, Devillard E, Heinrichs DE, Reid G, Mccormick JK. Inhibition of expression of a staphylococcal superantigen-like protein by a soluble factor from *Lactobacillus reuteri.* Microbiology. 2006;152(4):1155-1167. doi:10.1099/mic.0.28654-0.
11. Lee YD, Moon BY, Park JH, Chang HI, Kim WJ. Expression of enterotoxin genes in *Staphylococcus aureus* isolates based on mRNA Analysis. J Microbiol Biotechnol. 2007;17(3):461-467.
12. Kyriacou A, Tsimpidi E, Kazantzi E, Mitsou E, Kitrzalidou E, Oikonomou Y. Microbial content and antibiotic susceptibility of bacterial isolates from yoghurts. Int J Food Sci Nutr. 2008;59(6):512-525. doi:10.1080/09637480701565729.
13. Alomar J, Loubiere P, Delbes C, Nouaille S, Montel MC. Effect of *Lactococcus garvieae, Lactococcus lactis* and *Enterococcus faecalis* on the behavior of Staphylococcus aureus in microfiltered milk. Food Microbiol. 2008;25(3):502-508. doi:10.1016/j.fm.2008.01.005.
14. Kazemi Darsanaki R, Lalehrokhi M, Azizollahi M, Issazadeh KH. Antimicrobial activities of lactobacillus strains isolated from fresh vegetables. Middle-East J Sci Res. 2012;11(9):1216-1219.
15. Otero MC, Nader-Macias ME. Inhibition of Staphylococcus aureus by H2O2-producing *Lactobacillus gasseri* isolated from the vaginal tract of cattle. Anim Reprod Sci. 2006;96(1-2):35-46. doi:10.1016/j.anireprosci.2005.11.004.
16. Sameshima T, Magome C, Takeshita K, Arihara K, Itoh M, Kondo Y. Effect of intestinal Lactobacillus starter cultures on the behavior of Staphylococcus aureus in fermented sausage. Int J Food Microbiol. 1998;42(1):1-7.
17. Gonzalez-Fandos E, Otero A, Sierra M, Garcia-Lopez M, Prieto M. Effect of three commercial starters on growth of Staphylococcus aureus and enterotoxin (A-D) and thermonuclease production in broth. Int J Food Microbiol. 1994; 24(1): 321-327.
18. Troller J, Frazier C. Repression of *Staphylococcus aureus* by food bacteria. I. Effect of environmental factors on inhibition. Appl Microbiol. 1963;11(1):11-14.

19. Ammor S, Tauveron G, Dufour E, Chevallier I. Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility. 1. Screening and characterization of the antibacterial compounds. Food Control. 2006;17(6): 454-461. doi:10.1016/j.foodcont.2005.02.006.
20. Guglielmotti MD, Marco BM, Golowczyc M, Reinheimer AJ, Quiberonidel LJ. Probiotic potential of Lactobacillus delbrueckii strains and their phage resistant mutants. Int Dairy J. 2007;17(8):916-925.
21. Suskovi J, Kos B, Beganovi J, et al. Antimicrobial activity - the most important property of probiotic and starter lactic acid bacteria. Food Technol Biotechnol. 2010;48(3): 296-307.
22. Maira J, Medellin-Pena MJ, Mansel WG. Effect of molecules secreted by *Lactobacillus acidophilus* strain La-5 on *Escherichia coli* O157:H7 colonization. Appl Environ Microbiol. 2009; 75(4):1165-1172.
23. Wang H, Johnson R, Anand S, Griffiths MW. Probiotics affect virulence-related gene expression in *Escherichia coli* O157:H7. Appl Environ Microbiol. 2007;73(13): 4259-4267.
24. Dicks LMN, Botes M. Probiotic lactic acid bacteria in the gastrointestinal tract: health benefits, safety and mode of action. Benef Microbes. 2010;1(1):11-29.
25. Tomioka H, Sato K, Saito H. Effect of ofloxacin combined with Lactobacillus casei against Mycobacterium fortuitum infection induced in mice. Antimicrob Agents Chemother. 1990;34(4):632-636.
26. Ruijter JM, Ramakers C, Hoogaars WM, Karlen Y, Bakker O, van den Hoff MJ. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res. 2009; 37(6):202-207. doi: 10.1093/nar/gkp045.
27. Azizkhani M, Misaghi A, Basti AA, Gandomi H, Hosseini H. Effects of Zataria multiflora Boiss. Essential oil on growth and gene expression of enterotoxins A, C and E in *Staphylococcus aureus* ATCC 29213. Int J Food Microbiol. 2013;163(2-3):159-165. doi:10.1016/j.ijfoodmicro.2013.02.020.