ORIGINAL ARTICLE

Osong Public Health Res Perspect 2017;8(1):54–60
https://doi.org/10.24171/j.phrp.2017.8.1.07
eISSN 2233-6052

The Effect of *Lactobacillus acidophilus* PTCC 1643 on Cultured Intestinal Epithelial Cells Infected with *Salmonella enterica* serovar Enteritidis

Mona Moshiri[a], Mohammad Mehdi Soltan Dallal[a,b], Farhad Rezaei[c], Masoumeh Douraghi[a], Laleh Sharifi[d], Zahra Noroozbabaei[c], Mehrdad Gholami[e], Abbas Mirshafiey[f]

[a]Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[b]Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c]Department of Virology, Tehran University of Medical Sciences, Tehran, Iran
[d]Research Center for Immunodeficiencies, Tehran University of Medical Sciences, Tehran, Iran
[e]Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
[f]Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Objectives: Gastrointestinal disorders caused by *Salmonella enterica* serovar Enteritidis (*Ses*E) are a significant health problem around the globe. Probiotic bacteria have been shown to have positive effects on the immune responses. *Lactobacillus acidophilus* was examined for its capability to influence the innate immune response of HT29 intestinal epithelial cells towards *Ses*E. The purpose of this work was to assess the effect of *L. acidophilus* PTCC 1643 on cultured intestinal epithelial cells infected with *Ses*E.

Methods: HT29 cells were cultured in Roswell Park Memorial Institute medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were treated with *L. acidophilus* PTCC 1643 after or before challenge with *Ses*E. At 2 and 4 hours post-infection, we measured changes in the expression levels of *TLR2* and *TLR4* via real-time polymerase chain reaction.

Results: Treatment with *L. acidophilus* inhibited *Ses*E-induced increases in *TLR2* and *TLR4* expression in the infected HT29 cells. Moreover, the expression of *TLR2* and *TLR4* in cells that were pretreated with *L. acidophilus* and then infected with *Ses*E was significantly higher than that in cells infected with *Ses*E without pretreatment. Taken together, the results indicated that *L. acidophilus* had an anti-inflammatory effect and modulated the innate immune response to *Ses*E by influencing *TLR2* and *TLR4* expression.

Conclusion: Our findings suggested that *L. acidophilus* PTCC 1643 was able to suppress inflammation caused by *Ses*E infection in HT29 cells and reduce *TLR2* and *TLR4* expression. Additional in vivo and in vitro studies are required to further elucidate the mechanisms underlying this anti-inflammatory effect.

Key Words: *Lactobacillus acidophilus*, probiotics, *Salmonella*, gene expression

Corresponding author:
Mohammad Mehdi Soltan Dallal
E-mail: msoltandallal@gmail.com

This paper reports part of a research project approved by the Food Microbiology Research Center, Tehran University of Medical Sciences and Health Services (Contract No. 26627).

INTRODUCTION

Probiotic strains are live bacteria and yeasts that have beneficial effects on human health when ingested, particularly for the digestive system [1]. They are capable of changing the population of flora in the gut and preventing intestinal infections, gut cancer, and allergy, and they have specific immunomodulatory properties in the gut that are mediated by the repression of inflammatory mediators. Additionally, they can influence the properties of the intestinal bar-

rier [2,3]. The functional effects of probiotics are correlated with their capabilities to compete with pathogenic organisms for adhesion positions and adjust the host's immune response [4]. Among the many health benefits attributed to probiotics, their beneficial interactions with the immune system are supported by a growing corpus of in vivo and in vitro experiments as well as clinical evidence [5]. Lactic acid bacteria, such as lactobacilli and bifidobacteria, are usual residents of the human gastrointestinal tract and are commonly used in dairy products [6]. *Salmonella* is known as a major causative agent of food-borne illness in developed and developing countries [7]. *Salmonella enterica* serovar Enteritidis (*Ses*E) is the predominant cause of food-borne salmonellosis in many countries including Iran [8]. Previous studies demonstrated that *Lactobacillus* spp. have immunomodulatory effects and antagonistic effects against *Salmonella* infection both in vitro and in vivo [9–11]. Many of the effects of probiotic strains are mediated via immune regulation, especially through modulating the balance between pro- and anti-inflammatory cytokines [12]. Host pattern recognition receptors, such as Toll-like receptors (TLRs), play a key role in the recognition of pathogen components and the response to specific pathogen-associated molecular patterns that are associated with diverse microorganisms including bacteria, viruses, and fungi [13]. Hence, the induction of *TLR2* and *TLR4* after infection with intestinal bacteria may lead to the overexpression of inflammatory cytokines through the activation of their corresponding signaling pathways [14]. *Lactobacillus* strains are able to suppress the activation of *TLR2* and *TLR4* signaling. Furthermore, some probiotic strains have been shown to increase phagocytosis in phagocytic cells and modify cytokine production following interactions with TLRs in different cell populations, although the studies are limited to date and the results have sometimes been contradictory [15]. Thus, the purpose of this work was to assess the effect of the probiotic *L. acidophilus* PTCC 1643 on cultured intestinal epithelial cells infected with *Ses*E in vitro.

MATERIALS AND METHODS

1. Bacterial preparation

This study was conducted in the Microbiology Laboratory of the Department of Microbiology of Tehran University of Medical Sciences in Tehran, Iran, during 2014 and 2015. *L. acidophilus* PTCC 1643 was purchased from the Persian Type Culture Collection of the Iranian Research Organization for Science and Technology in Tehran, Iran. *L. acidophilus* was cultured in de Man, Rogosa, and Sharpe broth (Merck, Darmstadt, Germany) for 48 hours under a 5% CO_2 atmosphere at 37°C. Stock cultures were maintained at −20°C in 15% glycerol. *Ses*E ATCC 17036 was cultured in Luria–Bertani medium (Merck) at 37°C overnight [16].

2. Cell culture

In this study, the HT29 human colon adenocarcinoma cell line was purchased from the Cell Bank at the International Genetic and Biological Center (Tehran, Iran), and grown in 25 cm^2 cell culture flasks (Greiner Bio-one, Frickenhausen, Germany) in an incubator with 95% (v/v) humidified air containing 5% CO_2 at 37°C for 4–5 days. The cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640; HyClone, Logan, UT, USA) supplemented with 10% (v/v) inactivated fetal bovine serum and 1% (v/v) penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). A negative control (untreated HT29 cells) was used in all experiments.

3. Co-culture of *L. acidophilus* and SesE with HT29 cells

HT29 cells were seeded into 12-well plates at a density of 1×10^6 cells/well. After 2–3 hours of incubation, the culture medium was replaced with fresh RPMI 1640 medium (without penicillin/streptomycin). HT29 cells were co-incubated with *L. acidophilus* at a density of 1×10^7 colony-forming units (CFU)/well for 2 hours at 37°C under a 5% CO_2 atmosphere. In separate 12-well plates, HT29 cells were infected with *Ses*E at a density of 1×10^7 CFU/well for 2 hours at 37°C under a 5% CO_2 atmosphere. After incubation, the cells were washed twice with culture media (RPMI 1640 without penicillin/streptomycin and fetal calf serum) and 150 μL of *Ses*E (1×10^7 CFU/well) was added to a plate containing HT29 and *L. acidophilus*, then 150 μL of *L. acidophilus* (1×10^7 CFU/well) was added to a plate containing HT29 and *Ses*E. Plates incubated with either *L. acidophilus* or *Ses*E alone were also included in the experiment. In the final step, the plates were rotated gently for 10 seconds and then incubated at 37°C for 2–4 hours. After incubation, HT29 cells were collected via 5 minutes centrifugation at 5,200 × *g* at room temperature. Each cell pellet was washed twice with sterile phosphate-buffered saline (pH 6.7) to quantify the expression levels of *TLR2* and *TLR4*.

4. RNA extraction and cDNA synthesis

Total RNAs were extracted from HT29 cells using the QIAzol® Lysis Reagent (Qiagen, Hilden, Germany). The quantity and purity of the RNA were assessed by measuring the absorbance at 260 nm and its ratio relative to that at 280 nm using an ultraviolet spectrophotometer (PhotoBiometer; Eppendorf, Hamburg, Germany). In the next step, total RNA was treated with RNase-free DNase I (Thermo Fisher Scientific, Waltham, MA, USA) to eliminate DNA contaminants and its integrity was checked by

Table 1. Primers used in the study

Primer	Sequence (5′ to 3′)	Reference
TLR2 F	GCA GAA GCG CTG GGG AAT GG	[17]
TLR2 R	GGA TGC CTA CTG GGT GGA GAA	[17]
TLR4 F	GGT GGA AGT TGA ACG AAT GG	[17]
TLR4 R	CCA GCA AGA AGC ATCAGG TG′	[17]
ACTB F	CTG GAA GAT GGT GAT GGG AT	[9]
ACTB R	GGA TTT GGT CGT ATT GGG CG	[9]

ACTB, β-actin; *TLR*, Toll-like receptor.

electrophoresis on an agarose gel stained with GelRed™ (Biotium, Hayward, CA, USA). For real-time (RT) polymerase chain reaction (PCR) experiments, cDNA was synthesized from total RNA using the QuantiTect® Reverse Transcription kit (Qiagen) according to the manufacturer's recommendations. The primer sequences used for this study are shown in Table 1. The β-actin gene (*ACTB*) was used as a housekeeping reference gene.

5. Quantitative RT-PCR

To characterize the effects of *L. acidophilus* on the expression of *TLR2* and *TLR4*, RT-PCR was carried out using SYBR® Premix Ex Taq™ (Takara Biotechnology, Otsu, Japan) on a thermal cycler (StepOnePlus™; Applied Biosystems, Foster City, CA, USA) during 40 cycles. Briefly, 20 μL of reaction mixture contained 5 μL cDNA, 10 μL Power SYBR® Green PCR master mix (Applied Biosystems), 4 μl RNase-free water, and 0.5 μL each of forward and reverse primers. The PCRs were performed with 1 cycle at 95°C for 10 minutes for initial denaturation followed by 40 cycles at 95°C for 30 seconds and 60°C for 30 seconds, then a final extension step for 30 seconds at 72°C. The results were expressed as mean values averaged from three independent experiments. *ACTB* was used as an endogenous control. In all tests, a negative control was used to determine contamination. The relative quantity (RQ) of gene expression for the sample was calculated using the $2^{-\Delta\Delta Ct}$ method.

6. Statistical analysis

Statistical analyses were performed using the Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) analysis tool package. Unless otherwise stated, all results are the mean values ± standard deviation of two independent experiments with at least 3 replicates. Data from each experiment were analyzed by one-way analysis of variance. Differences at $p < 0.05$ were considered to be statistically significant. Differences at $p < 0.01$ were considered to be statistically significant.

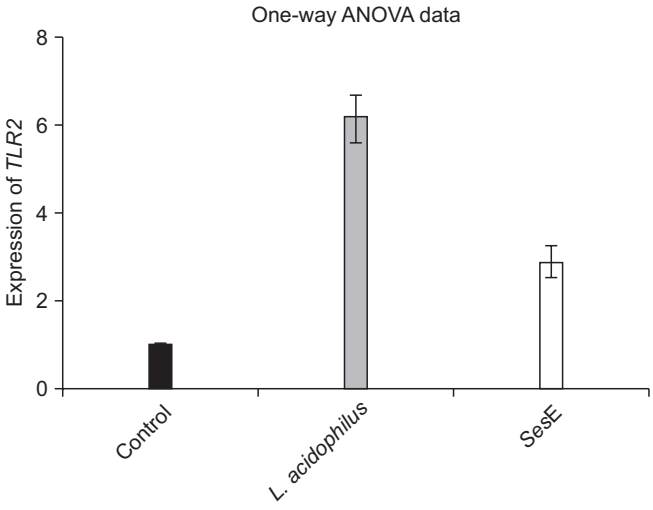

Figure 1. Effect of *Lactobacillus acidophilus* on *TLR2* mRNA expression in HT29 intestinal epithelial cells. Cells were treated with *L. acidophilus* and *Salmonella enterica* serovar Enteritidis (*Ses*E) individually. After 6 hours of incubation, real-time polymerase chain reaction was performed to measure *TLR2* mRNA expression levels. Untreated HT29 cells (filled histograms) were used as a control.

7. Ethics statement

Ethical approval was not required for the study, as there was no direct patient involvement and only cell cultures and bacterial strains were studied.

RESULTS

1. Effects of *L. acidophilus* and SesE on *TLR2* expression in HT29 cells

Analysis by RT-PCR revealed that the RQ of *TLR2* in HT29 cells after 6 hours of incubation with *Ses*E was 2.89, while that in the cells treated with *L. acidophilus* (without *Ses*E) was 6.13 ($p < 0.01$). Our results indicated that *TLR2* expression in HT29 cells exposed to *L. acidophilus* or *Ses*E was elevated compared with that of the control, and the increase in cells treated with *L. acidophilus* was higher than that in the cells infected with *Ses*E (Figure 1).

2. Effects of co-culture with *L. acidophilus* and SesE on *TLR2* expression in HT29 cells

When HT29 cells were incubated with *Ses*E for 2 hours and then treated with *L. acidophilus*, the RQ of *TLR2* expression after 2 hours treatment with *L. acidophilus* was 0.785, and after 4 hours treatment, the RQ decreased to 0.146. When the HT29 cells were treated with *L. acidophilus* and then incubated with *Ses*E, the RQs of *TLR2* expression after 2 and 4 hours incubation

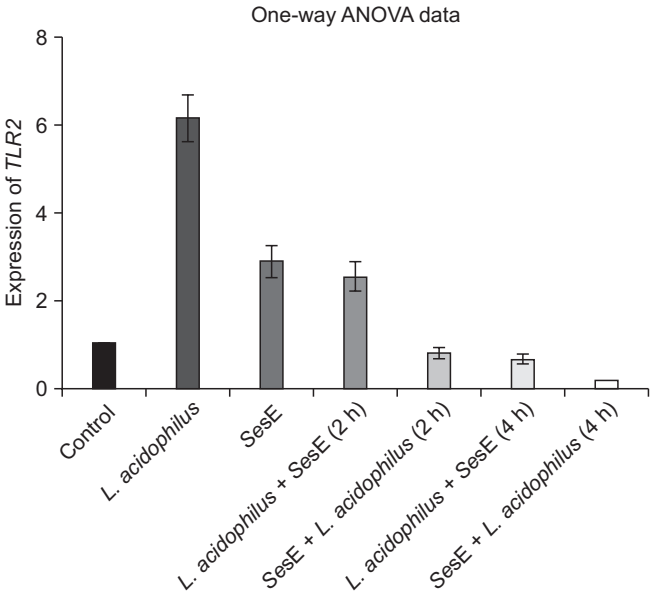

Figure 2. Effect of *Lactobacillus acidophilus* on *TLR2* mRNA expression in *Salmonella enterica* serovar Enteritidis (*Ses*E)-infected HT29 cells. Cells were incubated with *L. acidophilus* for 2 hours and then *Ses*E for 4 hours, or with *Ses*E for 2 hours and then *L. acidophilus* for 4 hours. At 2 and 4 hours after adding the second bacterium, real-time polymerase chain reaction was performed to measure *TLR2* mRNA expression levels. Untreated HT29 cells (filled histograms) were used as a control.

with *Ses*E were 2.55 and 0.650, respectively. The most notable of these findings was that *TLR2* expression in *Ses*E-exposed HT29 cells was markedly reduced after 4 hours of incubation with *L. acidophilus* (Figure 2).

3. Effects of *L. acidophilus* and SesE on *TLR4* expression in HT29 cells

TLR4 expression was investigated in HT29 cells exposed to either *L. acidophilus* or *Ses*E. The RQ of *TLR4* expression in the HT29 cells infected with *Ses*E was 1.489 after 6 hours incubation, while that in the cells treated with *L. acidophilus* was 3.97 ($p < 0.001$). These findings indicated that *L. acidophilus* increased the expression of *TLR4* in the HT29 cells to a greater extent than *Ses*E did (Figure 3).

4. Effects of co-culture with *L. acidophilus* and SesE on *TLR4* expression in HT29 cells

To further examine the effects of *L. acidophilus* and *Ses*E on *TLR4* expression in HT29 cells, RT-PCR was performed. When HT29 cells were incubated with *Ses*E for 2 hours and then treated with *L. acidophilus*, the RQs of *TLR4* expression at 2 and 4 hours after treatment with *L. acidophilus* were 0.623 and 0.414, respectively.

When HT29 cells were treated with *L. acidophilus* for 2 hours

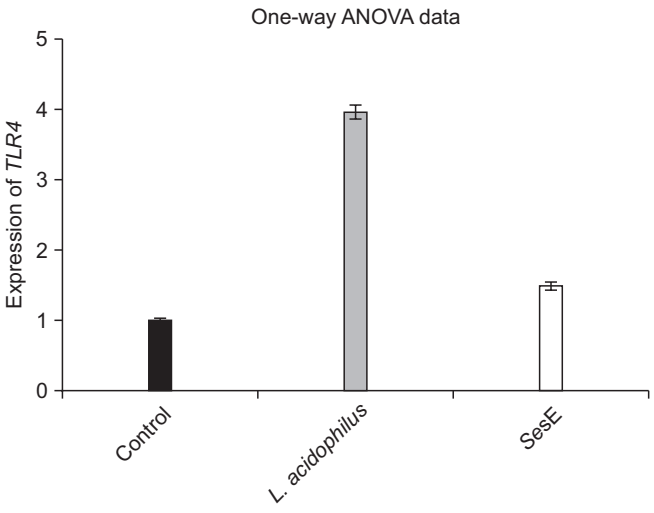

Figure 3. Effect of *Lactobacillus acidophilus* on *TLR4* mRNA expression in HT29 cells. Cells were treated with *L. acidophilus* and *Salmonella enterica* serovar Enteritidis (*Ses*E) individually. After 6 hours of incubation, real-time polymerase chain reaction was performed to measure *TLR4* mRNA expression levels. Untreated HT29 cells (filled histograms) were used as a control.

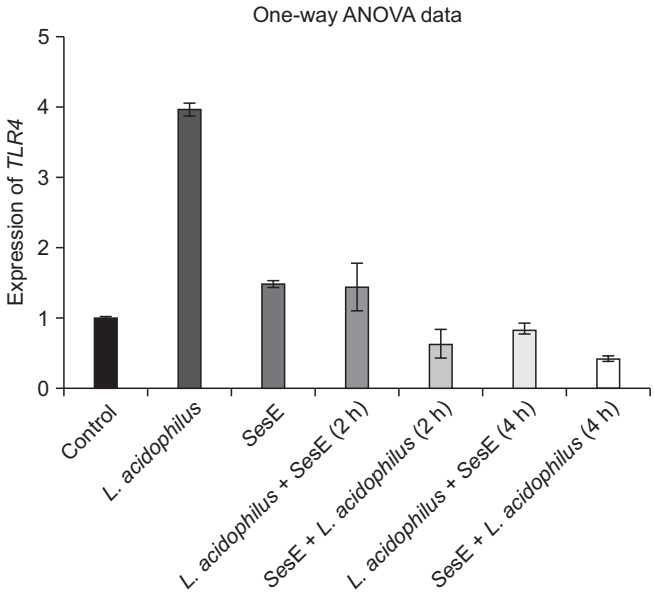

Figure 4. Effect of *Lactobacillus acidophilus* on *TLR4* mRNA expression in *Salmonella enterica* serovar Enteritidis (*Ses*E)-infected HT29 cells. Cells were incubated with *L. acidophilus* for 2 hours and then *Ses*E for 4 hours, or with *Ses*E for 2 hours and then *L. acidophilus* for 4 hours. At 2 and 4 hours after adding the second bacterium, RT-PCR was performed to measure *TLR4* mRNA expression levels. Untreated HT29 cells (filled histograms) were used as a control.

and then incubated with *Ses*E, *TLR4* expression significantly increased after 2 hours exposure to *Ses*E (RQ = 1.432) but was markedly reduced after 4 hours exposure to *Ses*E (RQ = 0.840).

Taken together, the results showed that the expression levels of *TLR2* and *TLR4* were reduced after treatment with *L. acidophilus* in HT29 cells pre-incubated with *Ses*E, and *TLR4* was downregulated more than *TLR2* (Figure 4).

DISCUSSION

Food-borne diseases caused by *Ses*E remain a major public health concern. *Ses*E is one of the predominant bacteria that cause human salmonellosis in many developing countries including Iran [8]. Recently, probiotics have been effectively used for the treatment and inhibition of enteric infections in humans [17]. Among the normal human gastrointestinal flora, *L. acidophilus* plays a key role in exerting inhibitory effects on the growth of obligate pathogens such as *Ses*E, *Staphylococcus aureus*, *Shigella dysenteriae*, and other microorganisms [18,19]. Hence, previous studies have shown that *L. acidophilus* possesses a strong anti-inflammatory activity [20,21].

Previous studies have reported conflicting results about the expression of *TLR2* in intestinal epithelial cells. Melmed et al [22] showed that intestinal epithelial cells were largely inattentive to bacteria that are recognized via *TLR2*. Otte et al [23] reported that intestinal epithelial cell lines showed reduced *TLR4* and *TLR2* expression during prolonged contact with TLR ligands (lipopolysaccharides and lipoteichoic acid). Furrie et al [24] reported that the expression of *TLR2* and *TLR4* was significantly increased in HT29 cells when they were cocultured with certain gram-positive bacteria, but gram-negative bacteria elicited no such change in expression. *TLR2* stimulation can protect the epithelial barrier, and it has been shown that the activation of *TLR2* plays an essential role in mediating resistance to bacterial invasion by enhancing the innate immune response [13]. Vizoso Pinto et al [25] reported that *TLR9* and *TLR2* were expressed by HT29 cells and their expression ratio changed after exposure to *Lactobacillus plantarum* BFE 1685, but was unchanged after incubation with *S. enterica* serovar Typhimurium only. Similar results were also obtained by Bermudez-Brito and coworkers [26]. In addition, other investigations have suggested that probiotics alone can promote the expression of *TLR2* and activate the innate immune response and immunostimulation [26–28].

In our study, the expression of *TLR2* significantly decreased in HT29 cells when they were treated with *L. acidophilus* either after or before infection with *Ses*E. Our observation that *L. acidophilus* modulates TLR gene expression is in agreement with several studies conducted in Germany, Spain, and France [25,26,29].

Our results revealed that *TLR4* expression in HT29 cells increased after treatment with *L. acidophilus* alone. Our findings agree with those previously reported by a study performed in Argentina [28]. In contrast, previous studies have shown that *TLR4* expression in Caco-2 cells decreased after treatment with lactic acid bacteria [30,31], whereas Miettinen et al [32] found that *Lactobacillus rhamnosus* GG did not influence *TLR4* expression.

In the present study, the expression of *TLR4* was decreased in HT29 cells when they were treated with *L. rhamnosus* either before or after infection with *Ses*E. The expression of both *TLR2* and *TLR4* was reduced after treatment with *L. acidophilus* in HT29 cells challenged with *Ses*E, but the downregulation of *TLR4* was higher.

L. acidophilus plays critical roles in regulating *TLR4* expression and inducing an anti-inflammatory response via decreasing inflammatory cytokine generation. Similar to our results, decreases in the expression of *TLR4* in response to probiotics and *Salmonella* have been reported by Bermudez-Brito et al [26] in Spain. Villena et al [33] reported that *Lactobacillus jensenii* TL2937 attenuates the inflammatory response triggered by the activation of *TLR4* in intestinal epithelial cells. Our results demonstrated that the use of *L. acidophilus* for the treatment of cells infected with *Ses*E significantly boosted innate immune responses. Overall, the obtained results suggested that *L. acidophilus* has strong immunomodulatory properties and can modulate the expression of *TLR2* and *TLR4* in HT29 intestinal epithelial cells challenged with *Ses*E. These observations support that *L. acidophilus* may have applications in the prevention and/or treatment of inflammatory diseases. Further in vivo and in vitro investigations are required to elucidate the mechanisms of these anti-inflammatory effects in future studies.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Vandenplas Y, Huys G, Daube G. Probiotics: an update. J Pediatr (Rio J) 2015;91:6-21. https://doi.org/10.1016/j.jped.2014.08.005
2. Thomas CM, Versalovic J. Probiotics-host communication: modulation of signaling pathways in the intestine. Gut Microbes 2010;1:148-

63. https://doi.org/10.4161/gmic.1.3.11712
3. Hemaiswarya S, Raja R, Ravikumar R, et al. Mechanism of action of probiotics. Braz Arch Biol Technol 2013;56:113-9. https://doi.org/10.1590/S1516-89132013000100015
4. Ambalam P, Raman M, Purama RK, et al. Probiotics, prebiotics and colorectal cancer prevention. Best Pract Res Clin Gastroenterol 2016;30:119-31. https://doi.org/10.1016/j.bpg.2016.02.009
5. Taverniti V, Guglielmetti S. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). Genes Nutr 2011;6:261-74. https://doi.org/10.1007/s12263-011-0218-x
6. Vidal K, Donnet-Hughes A, Granato D. Lipoteichoic acids from Lactobacillus johnsonii strain La1 and Lactobacillus acidophilus strain La10 antagonize the responsiveness of human intestinal epithelial HT29 cells to lipopolysaccharide and gram-negative bacteria. Infect Immun 2002;70:2057-64. https://doi.org/10.1128/IAI.70.4.2057-2064.2002
7. Zou W, Frye JG, Chang CW, et al. Microarray analysis of antimicrobial resistance genes in Salmonella enterica from preharvest poultry environment. J Appl Microbiol 2009;107:906-14. https://doi.org/10.1111/j.1365-2672.2009.04270.x
8. Eshraghi S, Dalall MMS, Fardsanei F, et al. Salmonella enteritidis and antibiotic resistance patterns: a study on 1950 children with diarrhea. Tehran Univ Med J 2010;67:876-82.
9. Rocha TS, Baptista AA, Donato TC, et al. Evaluation of in vitro and in vivo adhesion and immunomodulatory effect of Lactobacillus species strains isolated from chickens. Poult Sci 2012;91:362-9. https://doi.org/10.3382/ps.2011-01803
10. Servin AL. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol Rev 2004;28:405-40. https://doi.org/10.1016/j.femsre.2004.01.003
11. Feng J, Wang L, Zhou L, et al. Using in vitro immunomodulatory properties of Lactic acid bacteria for selection of probiotics against Salmonella infection in broiler chicks. PLoS One 2016;11:e0147630. https://doi.org/10.1371/journal.pone.0147630
12. Plaza-Diaz J, Gomez-Llorente C, Fontana L, et al. Modulation of immunity and inflammatory gene expression in the gut, in inflammatory diseases of the gut and in the liver by probiotics. World J Gastroenterol 2014;20:15632-49. https://doi.org/10.3748/wjg.v20.i42.15632
13. Majewska M, Szczepanik M. [The role of Toll-like receptors (TLR) in innate and adaptive immune responses and their function in immune response regulation]. Postepy Hig Med Dosw (Online) 2006;60:52-63. In Polish.
14. Villena J, Kitazawa H. Modulation of intestinal TLR4-inflammatory signaling pathways by probiotic microorganisms: lessons learned from Lactobacillus jensenii TL2937. Front Immunol 2014;4:512. https://doi.org/10.3389/fimmu.2013.00512
15. Soltan Dallal MM, Mojarrad M, Baghbani F, et al. Effects of probiotic Lactobacillus acidophilus and Lactobacillus casei on colorectal tumor cells activity (CaCo-2). Arch Iran Med 2015;18:167-72. https://doi.org/0151803/AIM.006
16. El-Nezami H, Kankaanpaa P, Salminen S, et al. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B1. Food Chem Toxicol 1998;36:321-6. https://doi.org/10.1016/S0278-6915(97)00160-9
17. Pagliaro G, Battino M. The use of probiotics in gastrointestinal diseases. Med J Nutrition Metab 2010;3:105-13. https://doi.org/10.1007/s12349-010-0008-9
18. Poppi LB, Rivaldi JD, Coutinho TS, et al. Effect of Lactobacillus sp. isolates supernatant on Escherichia coli O157: H7 enhances the role of organic acids production as a factor for pathogen control. Pesq Vet Bras 2015;35:353-9. https://doi.org/10.1590/S0100-736X2015000400007
19. Khanian SI, Mojgani N, Ahmedi MK. Characterization of partially purified bacteriocin like substance (BLIS) produced by probiotic Lactobacillus strains. Int J Enteric Pathog 2014;2:e17426. https://doi.org/10.17795/ijep17426
20. Toumi R, Soufli I, Rafa H, et al. Probiotic bacteria lactobacillus and bifidobacterium attenuate inflammation in dextran sulfate sodium-induced experimental colitis in mice. Int J Immunopathol Pharmacol 2014;27:615-27.
21. Wu Z, Pan DD, Guo Y, et al. Structure and anti-inflammatory capacity of peptidoglycan from Lactobacillus acidophilus in RAW-264.7 cells. Carbohydr Polym 2013;96:466-73. https://doi.org/10.1016/j.carbpol.2013.04.028
22. Melmed G, Thomas LS, Lee N, et al. Human intestinal epithelial cells are broadly unresponsive to Toll-like receptor 2-dependent bacterial ligands: implications for host-microbial interactions in the gut. J Immunol 2003;170:1406-15. https://doi.org/10.4049/jimmunol.170.3.1406
23. Otte JM, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology 2004;126:1054-70. https://doi.org/10.1053/j.gastro.2004.01.007
24. Furrie E, Macfarlane S, Thomson G, et al. Toll-like receptors-2, -3 and -4 expression patterns on human colon and their regulation by mucosal-associated bacteria. Immunology 2005;115:565-74. https://doi.org/10.1111/j.1365-2567.2005.02200.x
25. Vizoso Pinto MG, Rodriguez Gómez M, Seifert S, et al. Lactobacilli stimulate the innate immune response and modulate the TLR expression of HT29 intestinal epithelial cells in vitro. Int J Food Microbiol 2009;133:86-93. https://doi.org/10.1016/j.ijfoodmicro.2009.05.013
26. Bermudez-Brito M, Muñoz-Quezada S, Gomez-Llorente C, et al. Human intestinal dendritic cells decrease cytokine release against Salmonella infection in the presence of Lactobacillus paracasei upon TLR activation. PLoS One 2012;7:e43197. https://doi.org/10.1371/journal.pone.0043197
27. Vinderola G, Matar C, Perdigon G. Role of intestinal epithelial cells in immune effects mediated by gram-positive probiotic bacteria: involvement of toll-like receptors. Clin Diagn Lab Immunol 2005;12:1075-84. https://doi.org/10.1128/CDLI.12.9.1075-1084.2005
28. Castillo NA, Perdigón G, de Moreno de Leblanc A. Oral administration of a probiotic Lactobacillus modulates cytokine production and TLR expression improving the immune response against Salmonella enterica serovar Typhimurium infection in mice. BMC Microbiol 2011;11:177. https://doi.org/10.1186/1471-2180-11-177

29. Finamore A, Roselli M, Imbinto A, et al. Lactobacillus amylovorus inhibits the TLR4 inflammatory signaling triggered by enterotoxigenic Escherichia coli via modulation of the negative regulators and involvement of TLR2 in intestinal Caco-2 cells and pig explants. PLoS One 2014;9:e94891. https://doi.org/10.1371/journal.pone.0094891
30. Cammarota M, De Rosa M, Stellavato A, et al. In vitro evaluation of Lactobacillus plantarum DSMZ 12028 as a probiotic: emphasis on innate immunity. Int J Food Microbiol 2009;135:90-8. https://doi.org/10.1016/j.ijfoodmicro.2009.08.022
31. Wang S, Ng LH, Chow WL, et al. Infant intestinal Enterococcus faecalis down-regulates inflammatory responses in human intestinal cell lines. World J Gastroenterol 2008;14:1067-76. https://doi.org/10.3748/wjg.14.1067
32. Miettinen M, Veckman V, Latvala S, et al. Live Lactobacillus rhamnosus and Streptococcus pyogenes differentially regulate Toll-like receptor (TLR) gene expression in human primary macrophages. J Leukoc Biol 2008;84:1092-100. https://doi.org/10.1189/jlb.1206737
33. Villena J, Suzuki R, Fujie H, et al. Immunobiotic Lactobacillus jensenii modulates the Toll-like receptor 4-induced inflammatory response via negative regulation in porcine antigen-presenting cells. Clin Vaccine Immunol 2012;19:1038-53. https://doi.org/10.1128/CVI.00199-12