Original Article

APPLIED FOOD BIOTECHNOLOGY, 2016, 3 (3):170-176 pISSN: 2345-5357
Journal homepage: www.journals.sbmu.ac.ir/afb eISSN: 2423-4214

Elimination of Pathogen *Escherichia coli* O157:H7 in Ground Beef by a Newly Isolated Strain of *Lactobacillus acidophilus* during Storage at 5°C

Alireza Goodarzi[*], *Hrachya Hovhannisyan, Andranik Barseghyan*

Scientific and Production Center "Armbiotechnology" National Academy of Science, Yerevan, Armenia.

Abstract

Background and Objective: Constant use of limited number of lactic acid bacteria species in biopreservation can cause genetic degradation and or rising resistance against food pathogens or antimicrobial substances they produce. For this objective, a newly isolated strain of *Lactobacillus acidophilus* possessing high antimicrobial activity was evaluated as a candidate for use in bio-preservation.

Materials and Methods: Antibacterial activity was evaluated by agar disk diffusion method. Hydrogen peroxide amount was measured by Merckoquant Peroxide test strips. Microbiological analysis of the ground beef infected by *Escherichia coli* O157:H7 and treated by *Lactobacillus acidophilus* GH 201was done by plating of serial dilution in physiological saline on Tryptose agar.

Results and Conclusion: The cells (10^9 CFU ml^{-1}) of *Lactobacillus acidophilus* produced significant amount of antibacterial substances mainly hydrogen peroxide (28 and 30 µg ml^{-1}) in sodium phosphate buffer (0.2 M, pH 6.5) and LAPTg at 5°C during submerged cultivation with no growth, respectively. Submerged co-cultivation of *Escherichia coli* O157:H7 with lactobacilli in LAPTg broth at 5°C reduced the total number of the pathogen more than 2 log for 5 days. In case of solid state cultivation on agar-based medium, the maximum inhibitory zones on *Escherichia coli* O157:H7 lawn around the disks soaked by different amounts of washed *Lactobacillus acidophilus* cells appear for one-day cold exposition. The size of inhibition zone depends on the concentration of lactic acid bacteria cells. The cell suspension intended for treatment must contain 10^{8-9} CFU ml^{-1} of lactic acid bacteria. *Lactobacillus acidophilus* reduced the initial amount (2×10^5 CFU ml^{-1}) of *Escherichia coli* O157:H7 in ground beef up to 2 log for 5 days of solid-state co-cultivation. The application of *Lactobacillus acidophilus* bacteria expanded the shelf-life of ground beef due to inhibition of psychrophilic spoilage microorganisms.

Conflict of interests: The authors declare no conflict of interest.

Article Information

Article history
Received 25 March 2016
Revised 11 April 2016
Accepted 9 May 2016

Keywords
Biopreservation
E. coli O157:H7
Hydrogen peroxide
Lactobacillus acidophilus
Refrigerated temperatures

Correspondence to
Alireza Goodarzi
Scientific and Production Center Armbiotechnology
National Academy of Science, Yerevan, Armenia
P.O box: 0056
Tel: +374-94700166
Fax: +374-10654180
E-mail:
masivdo@yahoo.com

1. Introduction

Due to numerous outbreaks of food borne infections, food safety is one of the major concerns in public health. The safety of synthetic preservatives used in food is of concern for consumers so there is an increasing demand for natural food preservatives [1]. Biopreservation is an alternative method used to extend shelf-life and exclude resident pathogens in refrigerated products by introducing protective bacteria selected for their inhibitory activity towards undesirable microorganism. For these applications, LAB are usually chosen as they have the Generally Recognized As Safe (GRAS) status and produce a wide range of inhibitory compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins,

and thus, expanding shelf-life and increasing food safety [2-6]. The inhibitory actions of LAB toward food-borne pathogens and spoilage organisms in untreated foods must occur during the refrigerated storage [5]. The production of inhibitory compounds by these LABs continues during the entire storage period instead of a one-time reduction that occurs with antimicrobial substances [7,8]. It has been shown that at refrigerated temperatures, in the absence of growth, the most effectives are LABs, which produce hydrogen peroxide because at these temperatures, other antimicrobial substances are not synthesized or synthesized at very low levels [5-9]. Among the H_2O_2 producing LABs, strains of *Lactobacillus* (*L.*) *delbrueckii* subsp. *lactis* are mainly used in food preservation [10,11]; however, the use of limited number of LAB may cause decreasing of treatment efficacy due to accumulation of deleteious mutations and/or adapting of pathogens to antibacterial substances [2]. In order to enhance biopreservation efficacy, new LABs should be selected and appropriate methods should be developed for their cultivation and application to food [12-14]. Many strains of *L. acidophilus* were found to produce H_2O_2, but levels were relatively lower than those of other species [15]. Because hydrogen peroxide production plays the mayor role in elimination of pathogens at the refrigerator storage, it levels should be assessed for newly selected strains.

Most raw foods are contaminated with pathogenic and spoilage microorganisms. The known pathogen, *Escherichia* (*E.*) *coli* O157:H7, has become a significant worldwide cause of food borne outbreaks [16,18]. It is considered as one of the most serious known food borne pathogens due to severity of caused illnesses. Even 100 cells infective dose of *E. coli* O157:H7 may cause bloody hemorrhagic colitis, diarrhea, abdominal cramps, and in some cases, hemolytic uremic syndrome [16,19-21].

Ground beef products are common sources of *E. coli* O157:H7, and its reduction is an important concern in the beef industry [16,20]. Although many intervention technologies are applied to beef carcassses, ground beef processors currently do not have effective intervention steps for ground beef safe storage. There are only a few studies investigating the inhibitory effect of LAB on *E. coli* O157:H7 in ground beef products [5,22,23]. The objective of this research was to determine the main antibacterial products synthesized at cold storage in reach medium by newly isolated *L. acidophilus* GH 201, possessing high antimicrobial activity, and to evaluate whether its addition to raw ground beef would result in significant reduction of *E. coli* O157:H7 during the refrigerated storage.

2. Materials and methods

2.1. The bacterial cultures

The *L.acidophilus* GH 201 was isolated from Armenian healthy women vaginal microbiota, identified by API50 and RAPT-PCR in our laboratory, and deposited in the Armenian National Microbial Depository Center (MDC) under code MDC 9626 [24]. Food born pathogen *E. coli* O157:H7 MDC 5003 used in this study was from the MDC.

2.2. Media

LAPTg was made up of yeast extract 10g, peptone 15 g, tryptone 10 g, glucose 10 g, and Tween 1 ml per 1 liter of distillated water. For solid medium, 1.5 % Bacto-agar was included. Other media included Nutrient broth (Serva, Germany) and Tryptose agar (Merck, Germany). Fresh ground meat was purchased from a local butcher in Armenia and transported to the laboratory using a refrigerated box. In experiments, Sodium phosphate buffer (pH 6.5), saline solution 0.85% NaCl and Merckoquant Peroxide Test strips (Merck, Germany) were used.

2.3. Bacteriological analysis

Bacterial counts in liquid media were made using standard methods [25]. For enumeration of *E. coli* and lactobacilli in ground beef, 1 g treated meat sample was inoculated in 9 ml of sterile saline solution 0.85% w v^{-1}, which was homogenized, ten-fold serial dilutions were made and plated on Tryptose and MRS agars for determination of *E. coli* and LAB counts, respectively.

2.4. Hydrogen peroxide assay

H_2O_2 concentration was measured by Merckoquant Peroxide Test strips with measuring 0.5, 2, 5, 10, 25 and 1, 3, 10, 30 and 100 ranges according to the manufacturer's instructions [26].

2.5. Acidity Assay

The pH was measured at room temperature, using a digital pH meter (Hanna, Romania). Titratable acidity was expressed as a percentage of lactic acid, which was neutralized with 0.1 N NaOH until a pink color appeared in the presence of phenolphthalein.

2.6. Agar disk diffusion method

Agar disk diffusion method was used to evaluate the antimicrobial effect of LAB suspensions. *E. coli* O157: H7 culture are grown in NB broth for 18 h at 37°C, diluted to the concentration of 10^7 cells ml^{-1} and spread onto Tryptose agar. The paper discs (diameter, 5 mm) were soaked with LAB culture liquids and placed on the test culture lawn.

After 2 h exposition in cold, the plates were incubated at 37°C for 18 h and examined for size of clear inhibitory zones [27].

2.7. Quantification of antimicrobial activity of LAB in cold cultivation

Lactobacilli were grown in LAPTg broth for 18 h at 37°C, divided in four 10 ml aliquots, and

centrifuged at 12000 ×g for 10 min. Then sediment was resuspended in 10 ml of cold medium, sodium phosphate buffer (with or without glucose), LAPTg broth or saline solution and incubated at 5°C. Next every two days for 7 days, the antimicrobial activity, hydrogen peroxide amount, viable cells amount and pH of the cell cultures were determined [28].

2.8. Submerged co-cultivation of LAB along *E. coli* in nutrient broth (NB)

For evaluating the antagonistic activity of *L. acidophilus* GH 201 against *E. coli* O157:H7, the overnight culture was diluted 10^2 CFU ml^{-1} in 200 ml of fresh NB to obtain the cell concentration of approximately 10^5 CFU ml^{-1}. Then it was divided into two equal portions and supplied with *L. acidophilus* GH 201 in 1:100 and 1:10 ratios. Both samples were stored at 5°C and subjected to microbial analysis on days 0, 1, 3, 5 and 7.

2.9. Agar-based solid state co-cultivation of LAB with pathogen

The cells were harvested from the overnight cultures grown in LAPTg by centrifugation, and concentrated ten times in saline solution. Then two decimal dilutions were made, and the paper discs were soaked in the cell suspensions and placed on *E. coli* O157:H7 lawn on Tryptose agar. On the next day, the inhibitory zones around the disks were measured.

2.10. LAB antimicrobial activity determination in ground meat

200 g of freshly prepared commercial ground beef was obtaining from a local grocery. 150 g of this ground beef was inoculated with *E. coli* O157:H7 to obtain a pathogen concentration of 10^5 CFU g^{-1}; then it was divided into three equal portions. *L. acidophilus* GH 201 was prepared as described previously and added individually in two of the ground beef samples inoculated with *E. coli* O157:H7 at the final concentrations of 10^7 and 10^8 CFU g^{-1}, respectively. The third control portions of the ground meat with *E. coli* O157:H7 were processed in the same manner. All samples were mixed, packaged in vacuum polyethylene packets, kept at 5°C, and subjected for microbiological analysis on days 0, 1, 3, 5, 7 and 10 [6].

2.11. Statistical analysis

Statistical analysis was performed using the SPSS software for windows (Version 16) (SPSS Inc. Chicago, IL and USA). Mean and standard deviation was used to describe data. Fisher's range test was used to determine differences between the test groups. $p \leq 0.05$ were considered as significant. All experiments were done in triplicates.

3. Results and Discussion

3.1. Antibacterial substances production by washed *L. acidophilus* cells at 5°C

Laboratory experiments revealed that the hydrogen peroxide producing ability of LAB at limiting temperature is strongly dependent on the nutrition media composition used for their prior propagation, as well as the media for sub-cultivation at refrigeration temperatures. The largest amount of hydrogen peroxide formed at 5°C when the LAB cells were primarily propagated into rich medium and then transferred in sodium phosphate buffer [29, 30]. Production of antimicrobial compounds by *L. acidophilus* GH 201 cells was propagated overnight in LAPTg broth, and after washing, they were cultured in four media: phosphate buffer with and without glucose, physiological saline and LAPTg broth were studied during 4 weeks of storage at 5°C by disk diffusion method (Figures 1 & 2).

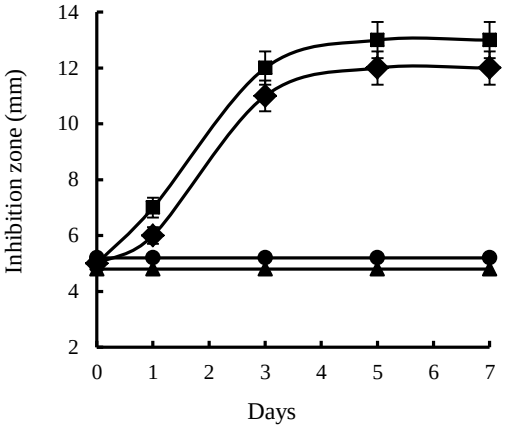

Figure 1. Zones of *E. coli* O157: H7 growth inhibition caused by *L. acidophilus* GH 201 washed cells inoculated in different media and stored at 5˚C. ● Phosphate buffer + glucose, ▲ Physiological saline, ♦ Phosphate buffer, ■ LAPTg broth.

The growth inhibition zones of *E. coli* O157:H7 around the discs, impregnated in the phosphate buffer without glucose and LAPTg bacterial suspensions, gradually increased during the 5 days of cold storage and reached to maximum diameters of 12 and 13 mm, respectively. In phosphate buffer with glucose and saline solution, no antibacterial activities were detected even during the entire 4 weeks of storage period. Our results are in agreement with the inhibition of *E. coli* O157:H7 and *S. aureus* cultures by the products of *L. acidophilus* cells inoculated in phosphate buffer without glucose obtained by Smith et al [5]. The cells inoculated in phosphate buffer containing 0% glucose produced significantly more H_2O_2 than those containing 1% or 10% glucose by Jaroni [28]. It's the first report about formation of inhibitory compounds in rich medium.

Figure 2. Inhibitory zones on *E. coli* O157:H7 caused by supernatants of *L. acidophilus* after 7 days of exposition at 5˚C in different media, 1: LAPTg broth; 2: phosphate buffer; 3: phosphate buffer+glucose, 4: physiological saline solution (0.85% w v^{-1}).

The inhibitory zone caused by the cells inoculated in LAPTg was larger than in phosphate buffer but was not significant (Figure 2).

3.2. Hydrogen peroxide production by *L. acidophilus* during storage at 5˚C

The dominant inhibitory factor produced by lactobacilli at refrigerating temperature was identified as hydrogen peroxide [10,31-33]. So in this study, production of hydrogen peroxide by *L. acidophilus* cells at 5°C storage was evaluated only in phosphate buffer and LAPTg. H_2O_2 accumulation by lactobacilli gradually increased and reached the maximum after five days of cold storage. The overall H_2O_2 amount in phosphate buffer and LAPTg was equal to 28 and 30 µg ml^{-1}, respectively. Thus, *L. acidophilus* GH 201 by hydrogen peroxide production is not inferior to known strains *L. delbrueckii* [28,32] (Figure 3). It was revealed that the rate of H_2O_2 production correlated with the growth of antibacterial activity in LAB supernatants (Figure 1), indicating that hydrogen peroxide is the main factor responsible for the inhibitory action produced by the lactobacilli (Figure 3).

Cell suspensions in phosphate buffer without glucose showed high accumulation of H_2O_2 in contrast to phosphate buffer containing glucose where undetectable amounts of H_2O_2 were produced. The supplemental glucose in phosphate buffer appeared to inhibit the production of H_2O_2 by the cells; this is in agreement with the findings of other authors who reported that low glucose concentration is better for H_2O_2 production [28,32,34]. But in our experiments, the high concentration of glucose (1% w v^{-1}) in LAPTg broth was not interfered with hydrogen peroxide production.

The antimicrobial effect of H_2O_2 is due to the oxidation of sulfhydryl groups causing denaturing of enzymes, and peroxidation of membrane lipids which increased membrane permeability. It is also a

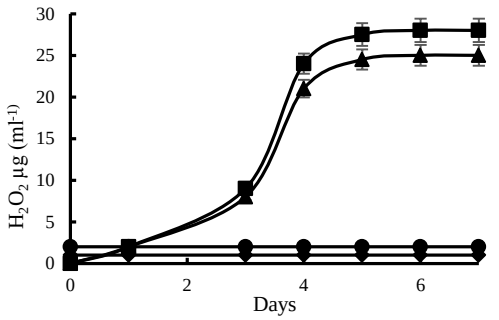

Figure 3. Kinetic of hydrogen peroxide production by *L. acidophilus* MDC 9626 at 5°C in different media. ● Phosphate buffer +glucose, ♦ Saline solution, ▲ Phosphate buffer, ■–LAPTg broth.

precursor for production of bactericidal free radicals e.g. superoxide (O^{-2}) and hydroxyl (OH^-), which candamage DNA [10,31]. H_2O_2 can have a strong oxidizing effect on cellular proteins and be produced using such enzymes as the flavoprotein oxidoreductases, NADH peroxidase, NADH oxidase and α-glycerophosphate oxidase [35, 36].

3.3. Cells viability and acidity changes during cold storage

The data of initial and final pH and the amount of viable *L. acidophilus* GH 201 cells during 7 days at cold storage are presented in Table 1.

No significant differences were found in the population levels of LAB cultures during over 4 weeks of storage at 5°C, indicating that LAB reproduction was not necessary for the inhibition of pathogens. These findings come in accordance with the observations of other authors who suggested that the production of inhibitory metabolites can occur by LAB during storage in the absence of growth [29,30]. Also, no significant changes of pH and titratable acidity in all of the media were detected, implying that antibacterial activity of LAB at refrigerator temperature is not caused by lactic acid synthesis.

3.4. Antagonistic action of *L. acidophilus* on *E. coli* O157:H7 in submerged co-cultivation at 5°C

L. acidophilus was added to nutrient broth along with *E. coli* O157:H7 in order to determine its antagonistic activity against the pathogen at 5°C. Two ratios of *E. coli* O157:H7 to *L. acidophilus* **(**1:100 and 1:10) were tested. The total number of *E. coli* O157:H7 cells in both treatments were determined on days 0, 1, 3, 5 and 7 by plating appropriate dilutions on Tryptose agar and incubation at 37°C for 24 h (Figure 4). On first day, there were no significant differences among the initial populations of of *E. coli* for all treatments (Figure 4). After 3 days of storage, number of *E. coli* O157:H7 was decreased for the treatments containing 1.1×10^8 of *L. acidophilus*. An additional significant decline (2 log) in the number of viable cells of *E. coli* was also observed after 5 days of storage.

Table 1. Cells viability and acidity in different media during storage at 5°C

	Day 0		After 7 days	
Incubation medium	pH	Viable cells, CFU ml^{-1}	pH	Viable cells, CFU ml^{-1}
LAPTg broth	6.5	6.3×10^8	6.5	5.2×10^8
PBS	6.8	6.3×10^8	6.8	5.1×10^8
PBS +glucose (1%)	6.3	6.3×10^8	6.3	5.8×10^8
Physiological solution	5.5	6.3×10^8	5.5	5.6×10^8

There was only 0.4 log decline in the treatment containing 1.4×10^7 CFU ml^{-1} of lactobacilli. It suggests that there must be sufficient number of LAB to have an antagonistic effect on pathogens. The number of viable cells of *E. coli* O157:H7 in the control was not significantly changed in the limiting temperature. These results are similar to data obtained by Brashears et al. [37].

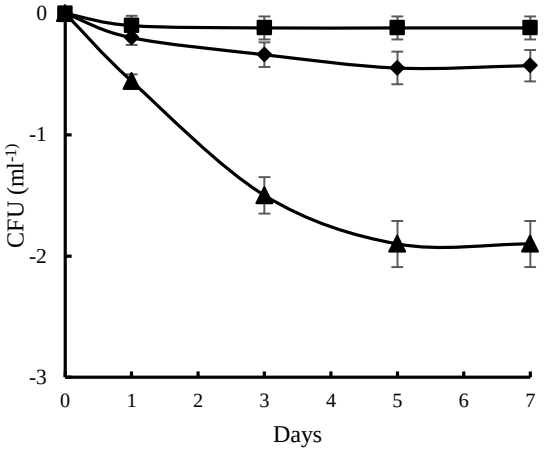

Figure 4. Kinetic of inactivation of *E coli* O157:H7 by *L. acidophilus* in NB submerged co-cultivation at 5°C.
(■) *E. coli* O157:H7, (♦) *E. coli* O157:H7+*L. acidophilus* GH 201 ratio (1:10), (▲) *E coli* O157:H7+*L. acidophilus* GH 201 ratio (1:100).

3.5. Solid state agar-based studies of LAB inhibitory effect on *E. coli* O157:H7

The study of the impact of LAB on *E. coli* on agar-based co-cultivation revealed a direct correlation between the number of cells and production of inhibitory substances (Figure 5). The larger inhibitory zones formed around the disks soaked in the 10^9 concentration of LABs cells (disk number 3). From reducing the amount of LAB cells included the disks, the zones of inhibition around them become significantly smaller reflected on the size of inhibitory zones because in aerobic environment where O_2 is available the production of H_2O_2 is noticeably higher and reach the maximum in shorter period.

Figure 5. Inhibition zone of *E. coli* O157:H7 growth on T agar around the disk soaked in *L. acidophilus* cells suspensions in physiological saline 10^9 (3), 10^8 (2), and 10^7 (1), CFU ml^{-1}

3.6. LAB inhibitory effect on *E. coli* O157:H7 in ground meat at 5°C

Cell suspension of *L. acidophilus* was tested in packaged stored ground meat for its ability to reduce the viability of *E. coli* O157:H7 during the storage temperature at 5°C. Fresh ground meat was inoculated with 10^5 CFU g^{-1} of *E. coli*. The trial samples were treated with *L. acidophilus* at a level of 10^7 and 10^8 CFU g^{-1} and stored at 5°C for 7 days in plastic vacuum bags. The samples were analyzed for *E. coli* O157:H7 survivors and lactic acid bacteria on days 1 to 7 (Figure 6).

Towards the end of ground meat vacuum storage, the quantity of *E. coli* O157:H7 reduced by 1-2 log depending of *L. acidophilus* ratio. But the LAB count in the treated and control samples after refrigeration storage of 7 days did not significantly change. Growth of LAB in a fresh meat held at refrigeration temperature is not desirable because it would lead to premature spoilage of the product [38-40]. Therefore, the selected strain *L. acidophilus* is able to synthesize antimicrobial compounds in amounts sufficient to inhibit the growth of pathogens and spoilages.

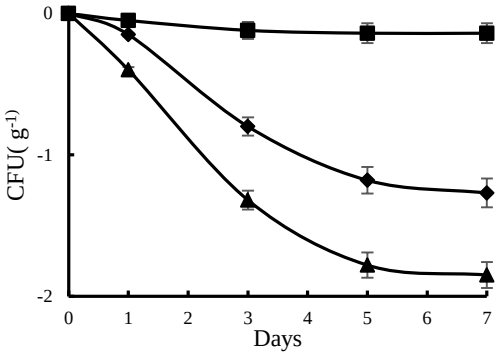

Figure 6: Kinetic of *E. coli* O157:H7 inactivation by *L. acidophilus* GH 201 in ground meat at 5°C. (■) *E. coli* O157:H7, (♦) *E. coli* O157:H7+10^7 *L. acidophilus* GH 201, (▲) *E. coli* O157:H7+10^8 *L. acidophilus* GH 201

The results of the experiments suggest that *L. acidophilus* (strain GH 201) has a potential to be used as a candidate culture in biopreservation method to improve the safety and extend the shelf-life of meat products in cold storage.

4. Conclusions

The cells of *L. acidophilus* GH 201 produced significant amount of antibacterial compounds (mainly hydrogen peroxide) at 5°C in the absence of growth. High concentrations of glucose have a suppressing impact on H_2O_2 production in sodium phosphate buffer, but had not suppressive effect in rich medium. In submerged and solid-state agar-based co-cultivation trials, *L. acidophilus* exerts inhibitory action against *E. coli* O157:H7 and significantly reduces the number of pathogen bacteria at the refrigeration temperatures when used in the final concentration more than 10^7 CFU g^{-1}. This study documented that *L. acidophilus* GH 201 eliminates more than 95% of the pathogen *E. coli* O157:H7 in ground meat under refrigerator conditions due to hydrogen peroxide and perhaps other antimicrobial substances production, and thus it can be used successfully in commercial applications, with a minimum of technological hurdles.

5. Acknowledgment

The authors are thankful to Dr. Gohar Grigoryan for handling of the strains used in this research, and SPC "Armbiotechnology" NAS for facilitation and technical support on this work.

6. Conflict of interests

The authors declare no conflict of interest.

References

1. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control 2014; 46: 412-429. doi.org/10.1016/.2014.05.047.
2. Davidson PM, Harrison MA. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. Scientific status summary. Food Technol. 2002; 56(11): 69-78.
3. Muhialdin BJ, Hassan Z. Screening of lactic acid bacteria for antifungal activity against *Aspergillus oryzae*. Am J Appl Sci. 2011; 8: 447-451. doi: 10.3844/ajassp.2011.447.451.
4. Dalié DKD, Deschamps AM, Richard F. Lactic acid bacteria Potential for control of mould growth and mycotoxins. A review. Food Control. 2010; 21: 370-380. doi:10.1016/j.foodcont.2009.07.011.
5. Smith L, Mann JE, Harris K, Miller MF, Brashears MM. Reduction of *Escherichia coli* O157:H7 and Salmonella in ground beef using lactic acid bacteria and the impact on sensory properties. J Food Prot. 2005; 68(8): 1587-1592.
6. Favaro L, Penna ALB, Todorov DD. Bacteriocinogenic LAB from cheeses: Application in biopreservation. Trend Food Sci Technol. 2015; 41: 37-48. doi:10.1016/j.tifs.2014.09.001.
7. Daly CW, Sandine E, Elliker PR. Interaction of food starter cultures and food-borne pathogens. *Streptococcus diacetilactis* versus food pathogens. J Milk Food Technol. 1972; 35: 349-357.
8. Daeschel MA. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol.1989; 43(1):164-167.
9. Dahiya RS, Speck ML. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J Dairy Sci. 1968; 51: 1568–1572. doi.org/10.3168/jds.S0022-0302 (68)87232-7.
10. Yap PS, Gilliland SE. Comparison of newly isolated strains of *Lactobacillus delbrueckii* subsp. *lactis* for hydrogen peroxide production at 5°C. J Dairy Sci. 2000; 83: 628–632. doi: http://dx.doi.org/10.3168/ jds.S0022-0302(00)74922-8.
11. Gilliland SE. Use of lactobacilli to preserve fresh meat. Proc Recip Meat Conf. 1980; 33: 54-58.
12. Jones RJ, Hussein HM, Zagorec M, Brightwell G, Tagg JR. Isolation of lactic acid bacteria with inhibitory against pathogens and spoilage organisms associated with fresh meat. Food Microbiol. 2008; 25: 228-234. doi:10.1016/j.fm.2007.11.001.
13. Kostrzynska M, Bachand A. Use of microbial antagonism to reduce pathogen levels on produce and meat products. A review. Can J Microbiol. 2006; 52: 1017-1026. doi:10.1139/W06-058.
14. Maragkoudakis PE, Mountzouris KC, Psyrras D, Cremonese S, Fischer J, Cantor MD, Tsakalidou E. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis.* Int J Food Microbiol. 2009; 130: 219-226. doi:10.1016/j.ijfoodmicro.2009.01.027.
15. Sanders ME, Klaenhammer TR. The scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. J Dairy Sci. 2001; 84(2): 319-331. doi.org/10.3168/jds.S0022-0302(01)74481-5.
16. Strachan NJC, Doyle MP, Kasuga F, Rotariu O, Ogden ID. Dose response modelling of *Escherichia coli* O157 incorporating data from foodborne and environmental outbreaks. Int J Food Microbiol. 2005; 103: 35-47. doi:10.1016/j.ijfoodmicro.2004.11.023.

17. Olorunshola ID, Smith SI, Cker AO. Prevalence of EHEC O157:H7 in patients with diarrhoea in Lagos. Nigeria. Acta Path Micro Im. 2000; 108: 761-763. doi: 10.1034/j.1600-0463.2000.d01-26.x.

18. Koyange L, Ollivier G, Muyembe JJ, Kebela B, Gouali M, Germani Y. Enterohemorrhagic *Escherichia coli* O157. Kinshasa. Emerg Infect Dis. 2004; 10: 968. doi: 10.3201/eid1005.031034.

19. Rivas M, Miliwebsky E, Chinen I, Roldán CD, Balbi L, García B, Fiorilli G, Sosa S, Kincaid J, Rangel J, Griffin PM. Characterization and epidemiologic subtyping of Shiga toxin-producing *Escherichia coli* strains isolated from hemolytic uremic syndrome and diarrhea cases in Argentina. Foodborne Pathog Dis. 2006; 3: 88-96. doi:10.1089/fpd.2006.3.88.

20. Meng J, Doyle MP, Zhao T, Zhao S. Enterohemorragic *Escherichia coli*, In: Doyle MP, Beuchat LR, Montville TJ (Eds). Food Microbiology: Fundamentals and Frontiers, 2nd edition, Washington, D.C. ASM Press. 2001; 193-213.

21. Blanco M, Blanco JE, Mora AJE, Rey J, Alonso MJM, Hermoso M, Hermoso J, Alonso MP, Dahbi G, González EA, Bernárdez MI, Blanco J. Serotypes, virulence genes and intimin types of Shigatoxin (verotoxin) producing *Escherichia coli* isolates from healthy sheep in Spain. J Clinical Microbiol. 2003; 41: 1351-1356. doi:10.1128/JCM.41.4.1351-1356.2003.

22. Holzapfel WH, Geisen R, Schillinger U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int J Food Microbiol. 1995; 24(3): 343-362. doi:10.1016/ 0168-1605(94)00036-6.

23. Salem AM. Biopreservation challenge for shelf-life and safety improvement of minced beef. Glo J Biotechnol Biochem. 2012; 7 (2): 50-60. doi: 10.5829 /idosi. gjbb. 2012. 7.2. 64112.

24. Hovhannisyan H, Grigoryan G. Evolution of adhesive and aggregative properties of lactic acid bacteria, isolated from healthy Armenian women vagina. Int J Med Pharm Sci. 2015; 6(3): 1-4.

25. Gerhardt P. Manual of methods for general bacteriology. Washington, D.C., American Society for Microbiology. 1981.

26. Wilks M, Wiggins R, Whiley A, Hennessy E, Warwick S, Porter H, Corfield A, Millar M. Identification and H_2O_2 production of vaginal lactobacilli from pregnant women at high risk of preterm birth and relation with outcome. J. Clin. Microbiol. 2004. 42: 713-717. doi:10.1128/JCM.42.2.713-717.2004.

27. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J Antimicob Chemother. 2008; 61(6): 1295-1301. doi:10.1093/jac/dkn090.

28. Jaroni D, Brashears MM. Production of hydrogen peroxide by *Lactobacillus delbrueckii* subsp. *lactis* as influenced by media used for propagation of cells. J Food Sci. 2000; 65(6): 1033-1036. doi:10.1111 /j.1365- 2621. 2000.tb09412. x.

29. Amézquita A, Brashears MM. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. J Food Prot. 2002; 65(2): 316-325.

30. Ruby JR, Ingham SC. Evaluation of potential for inhibition of growth of *Escherichia coli* O157:H7 and multidrug-resistant *Salmonella* serovars in raw beef by addition of a presumptive *Lactobacillus sakei* ground beef isolate. J Food Prot. 2009; 72: 251-259.

31. 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 antimicrobial compounds. Food Control. 2006; 17: 454-461. doi:10.1016/j.food cont. 2005. 02.006.

32. Villegas E, Gilliland SE. Hydrogen peroxide production by *Lactobacillus delbrueckii* subsp. *lactis* I at 5°C. J Food Sci. 1998; 63(6): 1070-1074. doi: 10.1111/j. 1365-2621.1998.tb15857.x.

33. Collins EB, Aramaki K. Production of hydrogen peroxide by *Lactobacillus acidophilus*. J Dairy Sci. 1980; 63: 353-357. doi:10.3168/jds.S0022-0302 (80) 82938-9.

34. Berthier F. On the screening of hydrogen peroxide-generating lactic acid bacteria. Lett Appl Microbiol. 1993; 16(3): 150-153. doi: 10.1111/j.1472-765X. 1993 tb01381. x.

35. Higuchi M, Shimada M, Matsumoto J, Yamamoto Y, Hayashi T, Kaga T, Kamio Y. Identification of two distinct NADH oxidases corresponding to H_2O_2-forming oxidases and H_2O_2 forming oxidase induced in *Streptococcus mutans*. J Gen Microbiol. 1993; 139: 2343-2351. doi: 10.1099/00221287-139-10-2343.

36. Rattanachaikunsopon P, Phumkhachorn P. Lactic acid bacteria: Their antimicrobial compounds and their uses in food production. Ann Biol Res. 2010; 1 (4): 218-228.

37. Brashears MM, Reilly SS, Gilliland SE. Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* 0157:H7 on refrigerated raw chicken meat. J Food Prot. 1998; 61: 166-170.

38. Gilliland SE, Speck ML, Morgan CG. Detection of *L. acidophilus* in feces of humans, pigs, and chickens. Appl Microbiol. 1975; 30: 541-545.

39. Senne MM, Gilliland SE. Antagonism action of cells of *Lactobacillus delbrueckii* subsp. *lactis* against pathogenic and spoilage microorganisms in fresh meat systems. J Food Prot. 2003; 66: 418-425.

40. Sakaridis I, Soultos N, Batzios Ch, Ambrosiadis I, Koidis P. Lactic acid bacteria isolated from chicken carcasses with inhibitory activity against Salmonella spp and *Listeria monocytogenes*. Czech J Food Sci. 2014; 32(1): 61-68. doi: 10.1016/j.anaerobe.2011.09. 009