http://dx.doi.org/10.22037/afb.v5i3.21127

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2018, 5 (3):173-183
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

The Use of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *Lactis* BB12, as Probiotics to Reduce the Risk of Food Poisoning in Minced Meat

Jawad Kadhim Isa[1], Seyed Hadi Razavi[2*]

1- Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran.

2- Center of Excellence for Application of Modern Technologies for Producing Functional Foods and Drinks and Bioprocess Engineering Laboratory (BPEL), Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran.

Abstract

Background and Objective: For the first time, a detailed study of the antimicrobial metabolites produced by probiotics was carried out as an alternative natural way of chemical additives and to support consumer health. The study was undertaken using *Lactobacillus acidophilus*, *Bifidobacterium animalis* ssp. *Lactis* BB12, as well as and antimicrobial products as protective cultures to reduce the risk of food poisoning in minced meat.

Materials and Methods: Samples of minced meat were stored at 4°C. The microbiological analysis of probiotics and pathogens bacteria was performed in days 0, 3, 7 and 14. In these periods, pH parameter and antimicrobial activity of the probiotics were analyzed.

Results and Conclusion: During the cold storage, the counts of inoculated pathogens in the minced meat samples in co-culture with each specific probiotic decreased at different levels; some had significant decrease ($p \leq 0.05$) and some others showed no significant change ($p > 0.05$). The probiotics displayed the ability to produce antibacterial substances (lactic acid, diacetyl and hydrogen peroxide) at different concentrations in the minced meat samples with significant increases ($p \leq 0.05$) until the end of cold storage. Probiotics exhibited the ability to produce bacteriocins. *Lactobacillus acidophilus* as a probiotic showed a significant effect as biopreservative against pathogens and was more effective when combined with *Bifidobacterium* BB12.

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

Article Information

Article history:
Received 21Apr 2018
Revised 11Jun 2018
Accepted 23Jun 2018

Keywords:
▪Antimicrobial
▪ Bacteriocin
▪ Biopreservation
▪ Cold storage
▪ Pathogenic bacteria

***Corresponding author:**
Seyed Hadi Razavi,
Department of Food Science and Technology
Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran.

Tel: +98-263-2248804
Fax: +98-263-2249453
E-mail: srazavi@ut.ac.ir

1. Introduction

Food spoilage, foodborne diseases, and food poisoning have always been among the main problems in food industry worldwide. Due to the increasing trend of meat consumption (meat products in particular), industrial manufacturers, have made great efforts to produce healthy products with good quality [1]. The use of antibiotics in concentrated animal production creates the additional risk of bacterial resistance, which comprises microbiological dangers instead of a strictly chemical remainder one [2]. Furthermore, pathogens are resistant to most common antibiotics; thus, there is a prerequisite to search for new antimicrobial agents and developing modern strategies to combat them [3]. Food preservation by natural and microbiological means is probably an appropriate method to decrease the occurrence of foodborne diseases, overcome the economic damage resulting from the microbial spoilage of foodstuffs, and meet the food requirements of the growing world population [4]. Primarily, consumers demand high quality, additive-free, safe, healthy, nutritious, vitamin-rich, minimally processed, fresh tasting, lightly preserved and functional foods with extended shelf life [5]. New approaches such as bio-preservation techniques have acquired increasing consideration as a measure of natural restriction of the growth of pathogenic and spoilage organisms in ready-to-eat foods. Some lactic acid bacteria (e.g. those normally correlated with meat products) produce antimicrobial proteins known as bacteriocins. Thus, bacteriocins might be regarded as natural preservatives in cooled meat products [6]. Bio-preservation refers to the prolonged shelf

life and enhancement of the microbial safety of food-related products by their natural or controlled microflora [7]. Lactic acid bacteria (LAB) are a major tool for bio-preservation of meat products since they involve the normal microflora of these foods and because of their ability to provide metabolic substances with antimicrobial influence against spoilage and pathogenic bacteria. Their traditional use in food production confirms their lack of pathogenicity; they are Generally Recognized as Safe (GRAS). Inhibition by LAB perhaps is the result of the effect of synergism among a number of mechanisms, including competition for production of nutrients, organic acids, hydrogen peroxide (H_2O_2), and antimicrobial constituents such as bacteriocin like substances [8].

Although red meat is an effective deliverer of nutrients (for example, protein, necessary amino acids, minerals and vitamins) and the source of energy, it is the most susceptible food and a very suitable substrate for the growth and multiplication of enteric pathogenic bacteria like *Staphylococcus* (*S.*) *aureus*, *Salmonella* (*S.*) spp., and *Escherichia* (*E.*) *coli* O157:H7, in addition to spoilage and LAB. Because of inadequate measures during the storage of fresh meat, post-processing, handling and crosscont-amination, it is necessary for maintaining it with high quality before consumption; hence, researchers are constantly looking for different methods to improve the quality and safety of such foods and prolonged their storage period [9]. Russell et al. [10] showed that Bifidobacterium as probiotic, have a long history of fit and safe use in fermented milk products, is combined with personal foods as food complements and have many positive influences on human health (for example, prevention of infection by pathogenic bacteria). Gálvez et al. [11] mentioned that bacteriocinogenic strains can be used either promptly as starter culture, as an adjunct, or as a co-culture in combination with a starter culture, or even as a protective culture (in particular in the case of non-fermented foods).

Until now, techniques to request improved food safety have been depending on chemical preservatives and antibiotics, or on the application of more drastic physical treatments (e.g. high or low temperatures). However, all of these agents either alone or in combination have limitations that can result in changes in the nutritional and physicochemical properties of food. The safety of artificial preservatives used in food is of concern for consumers; so, there is an increasing request for natural food preservatives. Recently, a novel scientific approach, namely "bio-preservation technology" has found many application in food production and gained more and more attention among the food industry researchers [12,13]. Therefore, the objective of this research is to use *Lactobacillus* (*L.*). *acidophilus* and *Bifidobacterium* (*B.*) *animalis* ssp. *lactis* BB-12 and their antimicrobial products as protective cultures to reduce the risk of food poisoning in minced meat.

2. Materials and Methods

2.1. Bacterial strains and culture situations

Probiotic bacterium *L. acidophilus* PTCC 1643 was kindly provided by Iranian Biological Resource Center, Tehran, Iran. Probiotic bacterium *B. animalis* ssp. *lactis* BB-12 PTCC 1736 was kindly provided by Persian Type Culture Collection (PTCC). The indicator organisms (pathogenic bacteria), which involve *S. Typhimurium* ATCC 14028 and *S. aureus* ATCC 29213, were obtained from the College of Veterinary, University of Tehran, Iran. The stock culture collection was maintained at 40°C in 40% glycerol. They were sub-cultured three times prior to use in an appropriate medium. From these, the probiotic bacteria were cultured in MRS broth (OXOID, CM0359 LTD., BASINGSTOKE, HAMPSHIRE, ENGLAND) and incubated at 37°C for 24 h under microaerophilic and anaerobic conditions for *L. acidophilus* and *B. animalis*, respectively. Both of the pathogenic bacteria were propagated in 10 ml of Brain Heart Infusion broth (QUELAB UK) and incubated at 37°C for 24 h. The cells were pelleted by centrifugation (6000 ×g for 20 min, 4°C), washed twice in 10 ml of 0.1% peptone water, and suspended in 5 ml of 0.1% peptone water. The absorbance wavelength (at 600 nm) was measured, and each suspension was diluted as necessary to obtain approximately equal cell densities of each isolate.

2.2. Source and processing of minced meat samples

Fresh red beef was purchased from the local market and transferred immediately and aseptically to the laboratory. Minced meat was prepared by crushing fresh red beef in a sanitary chopper. Portions of 200 g pieces were heated up at 100°C for 15 min, and subsequently, cooled down to room temperature. Then they were inoculated with $>1\times10^6$ CFU g^{-1} of pathogenic and probiotic bacteria. Models (20 g) were retained into a 250 ml glass sterile screw cap and put in storage for up to 14 days at 4°C. For microbiological analysis and pH measurement, the samples were mixed with 180 ml of sterile peptone water (0.15% peptone and 0.85% sodium chloride) solution and homogenized for 5 min while shaking vigorously [14]. Serial dilutions were surface plated on agar plates. The viable cell counts were expressed as log value. All experiments and analyses were replicated at least twice. The results offered are average of independent triplicates. The microbiological analyses were performed on days 0, 3, 7, and after day 14.

2.3. Growth conditions and enumeration methods

Probiotic bacteria were counted on MRS agar (1.8%), and the plates were transferred and incubated at 37°C for

48 h. *S. aureus* was enumerated after 48 h on Baird-Parker Agar Medium (Mumbai-400086, India) at 37°C. *S. typhimurium* was enumerated after 24 h on Mac Conkey Agar Oxoid (CM0007) at 37°C [15].

2.4. Determination of pH

The pH was measured in days 0, 1, 3, 7, and after day 14 with a pH electrode (GLp22, CRISON, EEC) in 10 ml aliquots taken from each of the minced meat samples after being calibrated with the specification buffers at pH 4.0 and 7.0.

2.5. Estimation of antibacterial activity

2.5.1. Estimation of lactic acid produced by the probiotic

Calculation of the generated lactic acid was controlled by titration of 25 ml of the minced meat sample with NaOH (0.1 N), which previously inoculated with each probiotic and filtered by filter paper. Indicator phenolphthalein (3 drops) was mixed. NaOH was next mixed gradually to the sample until a pink colour appeared. Each ml of 0.1 N NaOH is equal to 90.08 mg of lactic acid [16]:

$$\text{Titratable acidity of lactic acid} = \frac{\text{ml NaOH} \times \text{N NaOH} \times \text{M E} \times 100}{\text{Volume of sample used}}$$

Ml NaOH = Volume of NaOH consumed
N NaOH= Normality of NaOH
M E = Correspondent factor = 90.08 mg

2.5.2. Determination of diacetyl generated by the probiotic

Diacetyl was calculated by estimating 25 ml of the minced meat sample, which previously inoculated with each probiotic and filtered by filter paper. Hydroxylamine solution (7.5 ml) was utilized for residual titration. The containers were titrated with HCl (0.1 N) to a green-yellow end-point utilizing bromophenol blue as indicator. The correspondent point of HCl to diacetyl is 21.52 mg [16]:

$$\text{Diacetyl production} = \frac{(b - s)\,(100 - e)}{Vw}$$

b = Amount of ml of 0.1N HCl used in titration of the pattern
e = Correspondent factor = 21.52 mg
V_w= Volume of pattern
s = Number of ml of 0.1 N HCl used in titration of residue pattern

2.5.3. Determination of hydrogen peroxide (H_2O_2) produced by the probiotic

About 25 ml of diluted sulphuric acid H_2SO_4 was mixed to 25 ml of filtered of the minced meat sample, which previously inoculated with each probiotic and filtered by filter paper. Titration was completed with $KMnO_4$ (0.1N). Each ml corresponds to 1.70 mg of H_2O_2, and decolonization of the model was considered as the finish [16]:

$$H_2O_2 \text{ Concentration} = \frac{\text{ml } KMnO_4 \times NKMnO_4 \times \text{M.E} \times 100}{\text{ml} H_2SO_4 \times \text{Volume of sample used}}$$

ml $KMnO_4$ = Volume of $KMnO_4$ consumed
$NKMnO_4$ = Normality of $KMnO_4$
ml H_2SO_4 = Volume of H_2SO_4 mixed
M.E = Correspondent factor = 1.70 mg

2.5.4. Bacteriocin activity assay

The antimicrobial activity of the bacteriocin was routinely estimated by the agar-well diffusion assay (AWDA) method of the selected probiotic (*L. acidophilus*, and *B. animalis*) on *S. aureus*, and *S. typhimurium*, as indicator microorganisms. The probiotics were multiplied in MRS broth for 24 h at 37°C, and then the probiotic cultures were centrifuged. The pellets were discharged and the cell free culture supernatants (CFS) obtained were treated as follows:

(i) Cell free culture supernatants were left without any treatment.

(ii) Cell free culture supernatants treated with sodium-β-glycerophosphate (Merck) were combined with a final concentration of 2% (wv^{-1}) to eliminate the impact of lactic acid.

(iii) Cell free culture supernatants treated with sodium-β-glycerophosphate 2% (wv^{-1}) and 200 unit ml^{-1} of catalase (SIGMA, Catalase from bovine liver, 10.000 unit per mg protein). Cell free culture supernatants (CFS) of *B. animalis* was modified to pH 6.5-7.0 by 1 M NaOH to eliminate the impact of lactic and acetic acid produced by *B. animalis*.

Both supernatants (treated and non-treated) were sterilized by membrane filtration (0.2 µm-pore-size cellulose acetate filter) before being subjected to the antibacterial assay. The cell free culture supernatants were concentrated on a rotary evaporator (Heidoluph). The plates were filled with 20 ml of MRS 1.8% agar. Three wells (7.0 mm of diameter) were cut into the cool MRS agar plates and filled with 60 µl of the supernatants treated as above. The supernatants were permitted to dry for 1 h inside the wells at room temperature. The plates were covered with 10 ml of Brain Heart Infusion broth (0.8% agar) at 45°C, previously inoculated with 100 µl of an overnight culture of the selected indicator organisms (10^6-10^7 CFU ml^{-1}). The inoculated plates were incubated for 24 h at 37°C. The diameter of the inhibition zone was measured with calipers [17,18].

2.6. Statistical analysis

Data were analyzed using the analysis of variance procedures. All statistical analyses were performed using the SPSS software (ver. 22). Significant differences among the means were determined using Duncan's test ($p \leq 0.05$).

3. Results and Discussion

3.1. Effect of probiotic on pathogenic bacteria in minced meat

The effects of probiotics on the growth of *S. aureus* are presented in Table 1. Observations were made immediately after inoculation of the probiotics with indicator microorganisms in the flasks of minced meat during the 14 day of storage at 4°C. Table 1 shows that *L. acidophilus* and *Bifidobacterium* BB12 cultures have reduced the *S. aureus* population within 14 days at 4°C in the samples of minced meat at different levels. *S. aureus* decreased at the end of storage period in all probiotic formulations; the decrease was significant ($p \leq 0.05$) in the co-culture of *S. aureus* with *L. acidophilus*, and the average viable cell counts of *S. aureus* was reduced by 0.89 log cycle. *S. aureus* significantly decreased ($p \leq 0.05$) in co-culture with *L. acidophilus* plus *B. animalis*, and the average of viable cell counts of *S. aureus* was reduced by 2.83 log cycle at the end of storage time. *S. aureus* significantly increased ($p \leq 0.05$) when inoculated alone in the flasks of minced meat as the control without probiotic, and the average viable cell counts of *S. aureus* were increased by 1.39 log cycle at the same period. After 14 days of cold storage, the decrease of *S. aureus* when combined with *B. animalis* was not significant ($p > 0.05$), and the average of viable cell counts of *S. aureus* was reduced by 0.55 log cycle (Table 1). Table 1 also displays the change in pH values during 14 days of storage for the minced meat samples inoculated with *S. aureus* and each specific probiotic separately. There was a significant decrease ($p \leq 0.05$) in the mean pH values of all formulations at the end of storage period in comparison with the initial pH values at zero time.

Counts of probiotics in the samples of minced meat were increased at the end of storage period in co-culture with *S. aureus*, and the average viable cell counts of *L. acidophilus* significantly were increased ($p \leq 0.05$) by 0.55 log cycle, whereas the increase of *B. animalis* was not significant ($p > 0.05$) by 0.24 log cycle at the end of storage time (Table 2).

Table 1. Growth of *Staphylococcus aureus* together with different probiotic cultures in samples of minced meat at 37°C and the development of pH during storage period.

Staphylococcus aureus with Probiotic	Changes in average values (log mean CFU g^{-1} ± SD, n=3) of *Staphylococcus aureus*				pH of the minced meat Average values (mean± SD, n=3)				
	Zero time (0)	3 d	7 d	14 d	Zero time (0)	1 d	3 d	7 d	14 d
Staphylococcus aureus with *Lactobacillus acidophilus*	7.05 ± 0.05^{a}	6.88± 0.17^{a}	6.88± 0.03^{a}	6.16 ± 0.01^{b}	7.00± 0.04^{a}	6.97± 0.01^{a}	6.76± 0.00^{b}	6.59± 0.01^{c}	6.45± 0.03^{d}
Staphylococcus aureus with *Bifidobacterium* BB12	6.61 ± 0.32^{ab}	6.84± 0.06^{a}	6.64 ± 0.37^{a}	6.06 ± 0.31^{b}	6.93± 0.01^{a}	6.46 ± 0.02^{b}	6.27 ± 0.01^{c}	6.24± 0.00^{d}	6.12± 0.01^{e}
Staphylococcus aureus with *Lactobacillus acidophilus* plus *Bifidobacterium* BB12	9.37 ± 0.20^{a}	7.82± 0.97^{b}	6.84± 0.17^{bc}	6.54± 0.27^{c}	6.95± 0.01^{a}	6.52± 0.03^{b}	6.26± 0.01^{c}	6.19± 0.01^{d}	6.02± 0.03^{e}
Staphylococcus aureus alone (control)	6.26± 0.24^{c}	6.53± 0.20^{bc}	6.86± 0.15^{b}	7.65 ± 0.12^{a}					

Different letters in same row represent significant differences ($p \leq 0.05$).

Table 2. Growth of probiotic in co-culture with. *Staphylococcus aureus* in samples of minced meat at 37°C during storage period

Changes in average values (log mean CFU g^{-1} ± SD, n=3) of probiotic		
Period	*Lactobacillus acidophilus*	*Bifidobacterium* BB12
Zero time(0)	7.22 ± 0.03^{b}	8.55 ± 0.15^{a}
3 d	7.64 ± 0.04^{a}	8.64 ± 0.12^{a}
7 d	7.70 ± 0.04^{a}	8.67 ± 0.12^{a}
14 d	7.77± 0.12^{a}	8.79± 0.15^{a}

Different letters in same column represent significant differences ($p \leq 0.05$).

Table 3 displays that both probiotic cultures reduced the *S. typhimurium* population within 14 days at 4°C in the flasks of minced meat at different levels. *S. typhimurium* decreased at the end of storage period in all probiotic formulations; the decrease was significant ($p \leq 0.05$) in combination of *S. typhimurium* with *L. acidophilus*, and the average viable cell counts of *S. typhimurium* were reduced by 1.25 log cycle. *S. typhimurium* decreased significantly ($p \leq 0.05$) in co culture with a mixture of *L. acidophilus* and *B. animalis*, and the average viable cell counts of *S. typhimurium* were reduced by 1.37 log cycle at the end of storage time. *S. typhimurium* y increased significantly ($p \leq 0.05$) when inoculated alone in the flasks of minced meat as the control without probiotic, and the average viable cell counts of *S. typhimurium* were increased by 0.49 log cycle in the same period. After 14 days of cold storage, the decrease of *S. typhimurium* was not significant ($p > 0.05$) when combined with *B. animalis*, and the average viable cell counts of *S. typhimurium* were reduced by 0.04 log cycle (Table 3). The table also shows the change in pH values during 14 days of storage period for the minced meat samples inoculated with *S. typhimurium* and each specific probiotic separately. There were significant decreases ($p \leq 0.05$) in the mean pH values of all formulations at the end of storage period in comparison with the initial pH values at zero time (day 0). Counts of probiotics in the samples of minced meat were increased significantly ($p \leq 0.05$) at the end of cold storage in co-culture with *S. typhimurium*, and the average viable cell counts of *L. acidophilus* and *B. animalis* were increased by 0.54 and 0.62 log cycles, respectively (Table 4).

In the current study, the most inhibition of *S. aureus* was obtained by co-culture with *L. acidophilus*, and *S. aureus* in co-culture with the mixture of *L. acidophilus* plus and *B. animalis*. Reduce in the concentration of *S. aureus* at the end of storage period was related to the decrease of pH values and the increase of probiotics; counts (Tables 1 and 2).

The most inhibition of *S. typhimurium* was obtained by its co-culture with *L. acidophilus*, and then in co-culture with the mixture of *L. acidophilus* and *B. animalis*. The decrease in the concentration of *S. typhimurium* at the end of cold storage was associated with the reduction of pH values and the increase of probiotics' counts (Tables 3 and 4).

Table 3. Growth of *Salmonella typhimurium* together with different probiotic cultures minced meat at 37°C and the development of pH during storage period

	Changes in average values (log mean CFU g^{-1} ± SD, n=3) of *Salmonella typhimurium*				pH of the minced meat Average values (mean± SD, n=3)				
Salmonella typhimurium together with probiotic	Zero time(0)	3 d	7 d	14 d	Zero time(0)	1 d	3 d	7 d	14 d
Salmonella typhimurium together with *Lactobacillus acidophilus*	8.15± 0.06^{a}	7.65± 0.27^{ab}	7.32± 0.74^{ab}	6.90± 0.43^{b}	6.74 ± 0.02^{a}	6.45± 0.01^{b}	6.43± 0.01^{b}	6.34± 0.02^{c}	6.30± 0.01^{d}
Salmonella typhimurium with *Bifidobacterium* BB12	7.65 ± 0.06 ^{c}	8.38± 0.06 ^{a}	8.22± 0.05^{b}	7.61± 0.04^{c}	6.97 ± 0.01 ^{a}	6.19± 0.56^{b}	6.33± 0.01^{b}	6.16± 0.01^{b}	6.02± 0.03 ^{b}
Salmonella typhimurium with *Lactobacillus acidophilus* plus *Bifidobacterium* BB12	7.82± 0.01 ^{c}	8.39± 0.02 ^{a}	8.11± 0.21 ^{b}	6.45± 0.03^{d}	6.91± 0.01 ^{a}	6.32± 0.02^{b}	6.18± 0.01^{c}	6.15± 0.02^{c}	6.01± 0.01 ^{d}
Salmonella typhimurium alone (control)	8.37 ± 0.02^{b}	8.47± 0.07^{b}	8.78± 0.06^{a}	8.86± 0.04^{a}					

Different letters in same row represent significant differences ($p \leq 0.05$).

Table 4. Growth of probiotic in co-culture with *Salmonella typhimurium* in samples of minced meat at 37°C during storage period

Changes in average values (log mean CFU g^{-1} ± SD, n=3) of probiotic		
Period	*L .acidophilus*	*Bifidobacterium* BB12
Zero time(0)	7.78± 0.07^{c}	8.28 ± 0.20^{b}
3 d	7.94 ± 0.20^{bc}	8.59± 0.25^{ab}
7 d	8.13 ± 0.15^{ab}	8.74 ± 0.31 ^{a}
14 d	8.32 ± 0.07^{a}	8.90 ± 0.11 ^{a}

Different letters in same column represent significant differences ($p \leq 0.05$).

3.2. Antibacterial metabolites produced by probiotics in minced meat samples

In the current study, all probiotics growing in the minced meat samples and in the refrigerated storage conditions exhibited the ability to produce antibacterial substances (lactic acid, diacetyl, and hydrogen peroxide). The concentration of antibacterial substances produced by both probiotics in the samples of minced meat was significantly increased ($p \leq 0.05$) after 14 days of cold storage compared to the day 1.

After 14 days of refrigerated storage, the pH values of the minced meat samples containing probiotics in co-culture with pathogenic bacteria decreased to about 6.0 despite the production of organic acid by the probiotics responsible for the reduction of pH. According to Sivasankar [19], some foods have buffering capacity in that they resist change in pH, in general; the buffering capacity of meat is greater because of its higher protein content compared to vegetables, which lack buffering capacity.

The concentrations of lactic acid, hydrogen peroxide and diacetyl produced by *L. acidophilus* after the end of the storage period in the minced meat sample were greater than in the case of *B. animalis* (Figures 1, 2 and 3).

Figure 1 shows the ability of both probiotics to produce lactic acid when grown in minced meat samples during the refrigerated storage period. *L. acidophilus* produced 0.062 g 100 ml^{-1} of lactic acid, whereas *B. animalis* produced 0.058 g 100 ml^{-1} of lactic acid at the end of cold storage. According to Tharmaraj [20], organic acids (for example lactic and acetic acids) produced by LAB help to lower the pH and create an unfavorable environment for other organisms. The author showed that for many years, the hydrogen ion was believed to be associated with the antimicrobial effect. The author added that recently, bacteriostatic and bactericidal effects of these weak acids are found to be caused by their un-dissociated molecules, rather than the hydrogen ion. The un-dissociated acid molecules damage the pathogens through acidification of cytoplasm, proton motive force destruction, and damaging the active transport of nutrients across the membrane and causing sub-lethal injury [21]. Theron and Lues demonstrated that, under acidic conditions, the undissociated organic acids are supposed to ease crossing the microbial membrane to the cytoplasm [22]. As the cytoplasmic pH declines, the growth is suppressed, and cell death happens eventually.

Makras and Vuyst referred that the Bifidobacterium strains checked throughout the study exhibited effective antibacterial activity against *S. typhimurium* SL1344 and *E. coli* C1845. They attributed this activity tothe formation of organic acids, in particular acetic and lactic acids [23]. In general, Gram-negative bacteria were more susceptible to these organic acids. Waterman and Small concluded that the buffer capacity of minced beef has been revealed to enhance the survival of *S. typhimurium* on minced beef in a low pH environment [24]. Suskovic et al. reported the toxic effects of lactic and acetic acid as decrease of intracellular pH and dissipation of the membrane's potential [25].

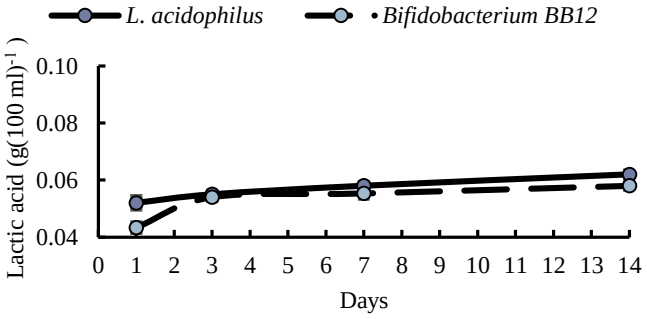

Figure 1. Lactic acid produced by probiotics during 14 days in minced meat, *L. acidophilus= Lactobacillus acidophilus*

Figure 2 displays the ability of both probiotics to produce diacetyl when grown in minced meat samples during the refrigerated storage at different concentrations. *L. acidophilus* produced 6.113 mg 100 ml^{-1} of diacetyl, whereas *B. animalis* produced 5.023 mg 100 ml^{-1} of diacetyl at the end of cold storage. Ray [26] showed that diacetyl was produced by some species of LAB in large quantities, especially in the metabolism of citrate. Suskovic et al. reported that diacetyl was more active against Gram-negative than Gram-positive bacteria [25]. Jay demonstrated that 15 cultures of Gram-negative bacteria were killed upon exposure to 258-344 µg ml^{-1} of diacetyl [27]. In our experiment, both probiotics showed the ability to produce diacetyl in the samples of minced meat at the end of cold storage at a range of 5.023-6.113 mg 100 ml^{-1} (Figure 2).

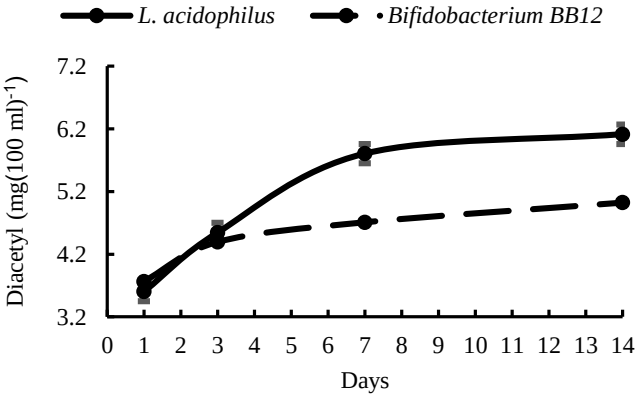

Figure 2. Diacetyl produced by probiotics during 14 days in minced meat. *L. acidophilus = Lactobacillus acidophilus.*

Figure 3 shows the ability of both probiotics to produce hydrogen peroxide when grown in minced meat samples during the refrigerated storage. *L. acidophilus* produced 0.067 mg 100 ml^{-1} of hydrogen peroxide whereas *B. animalis* produced 0.027 mg 100 ml^{-1} of hydrogen peroxide at the end of cold storage. According to Both et al., strains of *L. acidophilus* are microaerophilic, whereas *Bifidobacterium* subsp. Strains are anaerobic. Generally, these bacteria lack an electron transport chain, which results in imperfect reduction of oxygen to hydrogen peroxide [28]. Goodarzi et al. showed that hydrogen peroxide is a powerful oxidizing antimicrobial agent that oxidizes sulfhydryl groups and causes denaturing of enzymes, destruction of cell proteins and peroxidation of membrane lipids, ending with the enlargement of the membrane permeability. They added that hydrogen peroxide might also be a precursor for creation of bactericidal free radicals such as hydroxyl (OH^-) and superoxide (O_2^-) radicals, which can damage DNA [13].

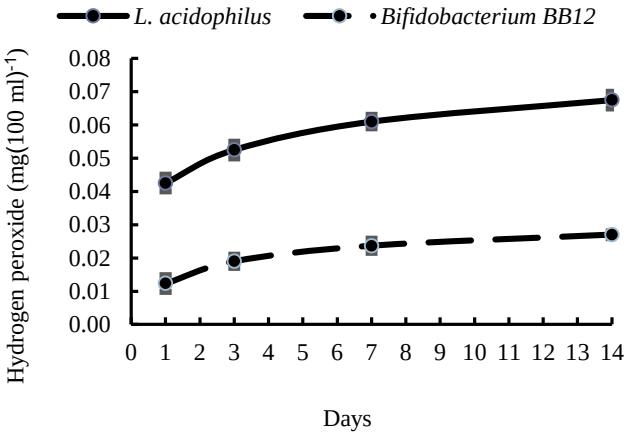

Figure 3. Hydrogen peroxide produced by probiotics during 14 days in minced meat .*L. acidophilus* = *Lactobacillus acidophilus.*

In the present experiment, the most efficient production of hydrogen peroxide in the samples of minced meat at the end of cold storage was obtained by *L. acidophilus* (Figure 3). Our results coincide to the findings of Klewicka and Libudzisz [29], who showed that hydrogen peroxide is another metabolic product of LAB, which demonstrates antibacterial activity. The authors claimed that Lactobacillus species have been identified as the most efficient producers of hydrogen peroxide among LAB, the most active being *L. acidophilus, L. plantarum*and *L. delbrueckii* spp. *Bulgaricus*. They also explained that hydrogen peroxide (10.0 µg ml^{-1}) can efficiently inhibit the growth of *S. aureus* [29].

In the current study, *L. acidophilus* and *B. animalis* were examined for exhibiting bacteriocin activity against two pathogenic bacteria (indicator microorganisms). Tables 5 and 6 show the ability of these two probiotics to produce bacteriocins or bacteriocin-like substances. The probiotics presented to have a wide inhibitory spectrum because they have the capability to inhibit both indicator microorganisms involved (i.e., *S. aureus* and *S. typhimurium).* According to Sifour et al., the inhibitory activity, which was noticed by the creation of observable and clear zones round the wells, can be probably attributed to the formation of many antimicrobial constituents like bacteriocin, organic acid and hydrogen peroxide [30]. In the current study, the efficacy of the inhibitory factors was examined under conditions, which excludes the probable influence of organic acids by modifying the pH of the cell-free supernatant (CFS) to 6.5-7.0 and of hydrogen peroxide by catalase treatment. When the cell-free supernatants were treated with Sodium glycerophosphate or 1M NaOH and 200 units ml^{-1} of catalase, the probiotics confirmed their activity against two indicator pathogenic bacteria at different levels. The diameters of the inhibition zones of the indicated organisms by the control cell-free supernatants, pH neutralized, and treated with catalase were extended from 7.20 to 12.06 mm. The greatest diameter (10.98 mm) was achieved with the control cell-free supernatants of *L. acidophilus* (without any treatment) against *S. typhimurium*, whereas the lowest diameter (7.20 mm) was obtained when treated with 1M NaOH and the cell-free supernatant (CFS) catalase of *B. animalis* against *S. typhimurium* (Tables 5 and 6).

Table 5. Inhibition zones (mm) (mean± SD, n=3) of pathogenic bacteria as indicator microorganisms by cell-free supernatant (CFS) of *Lactobacillus acidophilus*

Indicator microorganisms	Control of (CFS) without treatment	CFS with Sodium glycerophosphate (neutralize)[a]	CFS with Sodium glycerophosphate + Catalase enzyme[b]
Staphylococcus aureus	10.85 ±0.02	8.90 ± 0.02	8.20 ± 0.02
Salmonella typhimurium	10.98 ± 0.15	9.08 ± 0.07	7.63 ± 0.07

[a]CFS with pH neutralized to 6.5-7.0.
[b]CFS with pH neutralized to 6.5-7.0 and H_2O_2 eliminated.

Table 6. Inhibition zones (mm) (mean± SD, n=3) of pathogenic bacteria as indicator microorganisms by cell-free supernatant (CFS) of *Bifidobacterium* BB12

Indicator microorganisms	Control of (CFS) without treatment	CFS with 1M NaOH (neutralize)[a]	CFS with 1M NaOH+ Catalase enzyme[b]
Staphylococcus aureus	10.2 ± 0.03	8.54 ±0.09	7.71 ± 0.02
Salmonella typhimurium	8.80 ± 0.09	7.86 ± 0.19	7.20 ± 0.17

[a]CFS with pH neutralized to 6.5-7.0.
[b]CFS with pH neutralized to 6.5-7.0 and H_2O_2 eliminated

Cell-free supernatants (pH neutralized and treated with catalase) suppressed the growth of the indicator organisms, indicating that the bacteriocins produced by the probiotics may have antimicrobial activity. Gram-positive indicator organism is more susceptible to the bacteriocin of all probiotic strains than Gram-negative indicator organism (Tables 5 and 6). These results indicate that our probiotic bacteria had an inhibitory effect closely associated with Gram-positive bacteria. Our results are consistent with the findings of by Sifour et al., who reported that the resistance of Gram-negative bacteria could be attributed to the specific nature of the outer membrane [30].

Several studies have suggested that lactobacilli produce a wide range of antibacterial substances, comprising sugar catabolizes such as organic acids (e.g., acetic acid and lactic acid), oxygen catabolizes like hydrogen peroxide, and proteinaceous compounds such as bacteriocins [31, 32].

Schillinger et al. [14] noticed that in MRS broth, more than 99.9% of Listeria population was destroyed by bacteriocin during 24 h, while in the minced meat samples, the population of Listeria remained more or less constant. The authors demonstrated that a number of factors in the food pattern system might overlap with bacteriocin's activity. Sakacin A could adsorb to meat surface and fat constituent; hence, this might bring about its inactivation. Also the diffusion of bacteriocin probably is incomplete in minced meat. Correspondingly, Scott and Taylor pointed that nisin was considerably less efficient in heated meat medium comprising meat constituents than in trypticase peptone yeast glucose (TYPG) broth or brain heart infusion broth. So, they proposed that nisin connects to meat constituent [33]. Schillinger et al. concluded that in minced meat samples, there was an inactivation of bacteriocin after about 7 days at 8°C [14]. Pucci et al., who examined the efficiency of pediocin A from *Pediococcus* (*P.*) *acidilactici* in certain dairy products, reported the same observations. In half-and-half cream and in cheese sauce maintained at 4°C, there was also a recovery of *L. monocytogenes* after 7 days, showing an inactivation of bacteriocin [34].

Yang et al. [35]. Showed that the adsorption of all four bacteriocins onto the cells was strongly influenced by the pH of the suspending environment. Pediocin AcH was adsorbed by100% at pH 6.0-6.5, while at pH below 1.5, it was not adsorbed to either *P. acidilactici* LB 42-923 or *L. plantarum* NCDO 955.

According to Altuntas, organic acids could perform properly with bacteriocins as the rise in the net charge of bacteriocins at low pH might enable bacteriocin translocation across the cell wall. Furthermore, the solubility of certain bacteriocins might be enhanced at low pH as well, enabling diffusion. The author reported that incorporation of two or more bacteriocins may also give encouraging results, especially if the bacteriocins belong to diverse category schemes directing different cellular constituents [4]. Our results are consistent with previous studies in this regard. The number of pathogenic bacteria decreased at the end of storage period at different levels, some of which were significant ($p \leq 0.05$) and some not ($p > 0.05$). This indicates that the activity of bacteriocin perhaps diminishes by the connection of the bacteriocin molecules to the food constituents (mainly the fat matrix) in the minced meat samples, or adsorb onto the producer cells at pH values about 6.0 at the end of cold storage. At the same time, the use of a mixture of probiotics (*L. acidophilus* plus *B. animalis*) is more effective against the pathogenic bacteria compared to using the probiotic alone (Tables 1 and 3). Some researchers have evaluated the potential of some *Lactobacillus* and *Bifidobacterium* subsp. and their metabolic activity to control pathogens and spoilage microorganisms In vitro and in food systems. They found that the capability of the selected probiotics to inhibit the growth of several pathogens such as *S. aureus* and pathogenic *E. coli* O157:H7 In vitro and their potential mechanism of action are specific to a particular strain [36,37]. When evaluating the antimicrobial producing probiotics in minced meat samples or bio-preservation, one must bear in the memory that meat and meat products are complicated models with an amount of factors affecting microbial growth and metabolite manufacture, Our results are consistent with the results of these studies, showing the ability of probiotics to produce antibacterial substances (lactic acid, hydrogen peroxide, diacetyl and bacteriocin) and thus inhibit the growth of pathogenic bacteria in minced meat samples during cold storage. Meat and meat products are complicated models with amount of factors affecting microbial growth and metabolite manufacture.

Rossland et al. reported that biological models are complicated and LAB delay the growth of spoilage and pathogenic bacteria not merely by generating a diversity of antimicrobial substances, but also by competing for nutrients. The authors added that when the LAB is at extensively greater concentrations than the pathogenic bacteria in the existent co-cultures [38]. It is possible that the fast growth of a large population of LAB could retard the growth of other organisms easily by the intake of the most readily assimilation nutrients and co-factors, or even by physical occupation of the existing place.

Prudêncio et al. showed that temperature treatments might support disturbance in the outer membrane; in both cases, the low and high temperatures prefer the act of bacteriocins. A decrease in temperature encourages the alteration in the constitutes of the outer membrane. These changes make the bacteriocin be accessible to the cell, permitting the bacteriocin to perform on *S. Typhimurium* and *E. coli* at cooling temperatures. The refrigerating practice only permits the efficient sensitization of Gram-negative bacteria to the performance of bacteriocins; once the temperature declines speedily, since there is not sufficient time for the reformation of the outer membrane, this adjusts its penetrability [39]. The synergy between different of these preservative factors (hurdles) such as using a probiotic as alone or a mixture of both of probiotic at the same time with low temperature (4°C) of storage, it is possible to participate by retarding the growth of pathogenic bacteria, and ultimately to reducing of food poisoning in minced meat.

4. Conclusion

LAB has great potential for use in biopreservation because of their "GRAS" status. They are widely used in food industry as starter cultures, co-cultures incorporated with the primary starter cultures, or bio-protective cultures in a wide range of food and food products since earliest times without any safety risk. The production of a certain antimicrobial metabolites in laboratory media by LAB does not imply its effectiveness in a food model. Taking into consideration that minced meat is a complex system with an amount of factors affecting microbial growth and metabolite formation. However, *L. acidophilus* as probiotic had a significant role as a biopreservative in inhibiting the pathogenic bacteria in minced meat samples during cold storage and was more effective when combined with *Bifidobacterium* BB12.

5. Acknowledgements

The authors would like to thank the Department of Food Science and Technology, Agricultural Campus, University of Tehran for the support offered for the current work.

6. Conflict of Interest

The authors declare no conflict of interest.

References

1.Kheyri A, Fakhernia M, Haghighat-Afshar N, Hassanzadazar H, Kazemi-Ghoshchi B, Zeynali F, Asadzadeh J, Rahmanpour F, Bahmani M. Microbial contamination of meat products produced in the factories of west Azerbaijan province, north-west of Iran. Global Veterinaria. 2014; 12(6):796-802.
doi: 10.5829/idosi.gv.2014.12.06.83145

2. Castellano P, Belfiore C, Fadda S, Vignolo G. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. Meat Sci. 2008; 79(3): 483-499.
doi: 10.1016/j.meatsci.2007.10.009

3. Naghmouchi K, Baah J, Hober D, Jouy E, Rubrecht C, Sane F, Drider D. Synergistic effect between colistin and bacteriocins in controlling Gram-negative pathogens and their potential to reduce antibiotic toxicity in mammalian epithelial cells. Antimicrob Agents Chemother.2013; 57(6): 2719-2725.
doi: 10.1128/AAC.02328-12.

4. Altuntas EG. Bacteriocins: A Natural Way to Combat with Pathogens. In: Méndez-Vilas A. Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education, Formatex Research Center, Spain, 2013:1007-1015.

5. Sarika AR, Lipton AP, Aishwarya MS. Bacteriocin production by a new isolate of *Lactobacillus rhamnosus* GP1 under different culture conditions. Adv J Food Sci Technol. 2010; 2(5):291-297.

6. Winkowski KA, Crandall AD, Montville TJ. Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in beef systems at refrigeration temperatures. Appl Environ Microbiol.1993; 59(8): 2552-2557.

7. Zhou GH, Xu XL, Liu Y. Preservation technologies for fresh meat-a review. Meat Sci. 2010; 86(1): 119-128.
doi:10.1016/j.meatsci.2010.04.033

8. Castro MP, Cayre ME, PalavecinoPrpich NZ, Rivas FP. Control of Pathogens in Cooked Meat Products: The Beneficial Role of Lactic Acid Bacteria. In: Mendez-Vilas A. The Battle against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs, Formatex Research Center Spain, 2015:310-318.

9. AlizadehSani M, Ehsani A, Hashemi M. Whey protein isolate/cellulose nanofibre/TiO_2 nanoparticle/rosemary essential oil nanocomposite film: Its effect on microbial and sensory quality of lamb meat and growth of common foodborne pathogenic bacteria during refrigeration. Int J Food Microbiol. 2017; 251: 8-14.
doi:10.1016/j.ijfoodmicro.2017.03.018

10. Russell DA, Ross RP, Fitzgerald GF, Stanton C. Metabolic activities and probiotic potential of bifidobacteria. Int J Food Microbiol. 2011; 149(1):88-105.
doi:10.1016/j.ijfoodmicro.2011.06.003

11. Galvez A, Abriouel H, Lopez RL, Omar NB. Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol. 2007; 120(1-2): 51-70.
doi:10.1016/j.ijfoodmicro.2007.06.001

12. Amin RA. Effect of bio preservation as a modern technology on quality aspects and microbial safety of minced beef. Global J Biotechnol Biochem. 2012; 79(2):38-49. doi: 10.5829/idosi.gjbb.2012.7.2.64154

13. Goodarzi A, Hovhannisyan H, Barseghyan A. Elimination of pathogen *Escherichia coli* O157: H7 in ground beef by a newly isolated strain of *Lactobacillus acidophilus* during storage at 5°C. Appl Food Biotechnol. 2016; 3(3):170-171. doi: 10.22037/afb.v3i3.11799

14. Schillinger U, Kaya M, Lücke FK. Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J Appl Bacteriol. 1991; 70(6):473-478. doi: 10.1111/j.1365-2672.1991.tb02743.x

15. Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E. Probiotic potential of Lactobacillus strains isolated from dairy products. Int Dairy J. 2006; 16(3):189-199. doi:10.1016/j.idairyj.2005.02.009

16. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists, 25th Edition. AOAC, Washington D.C. 2000.

17. Buntin N, Chanthachum S, Hongpattarakere T. Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. Songklanakarin J Sci Technol. 2008; 30(1):141-148

18. Nespolo CR, Brandelli A. Production of bacteriocin-like substances by lactic acid bacteria isolated from regional ovine cheese. Braz J Microbiol. 2010; 41(4):1009-1018. doi: 10.1590/S1517-83822010000400020

19. Sivasankar B. Food Processing and Preservation. 6th edition. PHI Learning Private Limited, New Delhi, 2009: 120-132.

20. Tharmaraj N. Inhibitory Substances Produced by Probiotic Bacteria for Control of Food-Borne Pathogenic and Spoilage Microorganisms in Dips. Master of Science, Victoria University of Technology, Victoria Australia, 2004.

21. Lucke FK. Traditional Preservation Technologies. In: Zeuthen P, Bogh-Sorensen L. Food Preservation Techniques. Woodhead Publishing, Cambridge England, 2003.

22. Theron MM. Lues JFR. Organic Acids and Food Preservation, Talor and Francis Group, LLC. New York, USA, 2011: 117-149.

23.Makras L, De Vuyst L. The In vitro inhibition of Gram-negative pathogenic bacteria by bifidobacteria is caused by the production of organic acids. Int Dairy J. 2006; 16(9):1049-1057. doi:10.1016/j.idairyj.2005.09.006

24. Waterman SR, Small PL. Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain solid food sources. Appl Environ Microbiol. 1998; 64(10): 3882-3886.

25. Suskovic J, Kos B, Beganovic J, Pavunc AL, Habjanic K, Matoc S. Antimicrobial activity the most important property of probiotic and starter lactic acid bacteria. Food Technol Biotechnol. 2010; 48(3):296-307.

26. Ray B. Fundamental Food Microbiology. 3rd Edition, CRC Press, Boca Raton, Florida, 2005: 225-242.

27. Jay JM. Antimicrobial properties of diacetyl. Appl Environ Microbiol. 1982; 44(3): 525-532.

28. Both E, Gyorgy E, Abraham B, Lanyi S. Beneficial Effects of Probiotic Microorganisms. A review. Acta University Sapie Aliment. 2011; 4:44-58.

29. Klewicka E, Libudzisz Z. Antagonistic activity of *Lactobacillus acidophilus* bacteria towards selected food-contaminating bacteria. Pol J Food Nutr Sci. 2004; 13(2):169-174.

30. Sifour M, Tayeb I, Haddar HO, Namous H, Aissaoui S. Production and characterization of bacteriocin of *Lactobacillus plantarum* F12 with inhibitory activity against *Listeria monocytogenes*. J Sci Technol. 2012; 2(1):55-61.

31. Magnusson J, Strom K, Roos S, Sjogren J, Schnurer J. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. FEMS MicrobiolLett. 2003; 219(1):129-135. doi: 10.1016/S0378-1097 (02)01207-7

32. Abdel-Daim A, Hassouna N, Hafez MM, Ashor MS, Aboulwafa M. Antagonistic activity of Lactobacillus isolates against *Salmonella typhi* in vitro. Biomed Res Int. 2013; 2013: 1-13. doi:10.1155/2013/680605

33. Scott VN, Taylor SL. Effect of nisin on the outgrowth of *Clostridium botulinum* spores. J Food Sci. 1981; 46:117-126.

34. Pucci MJ, Vedamuthu ER, Kunka BS, Vandenbergh PA. Inhibition of *Listeria monocytogenes* by using bacteriocin PA-1 produced by Pediococcus acidilactici PAC 1.0. Appl Environ Microbiol. 1988; 54:2349-2353.

35. Yang R, Johnson MC, Ray BI. Novel method to extract large amounts of bacteriocins from lactic acid bacteria. Appl Environ Microbial.1992; 58: 3355-3359

36. Tejero-Sariñena S, Barlow J, Costabile A, Gibson GR, Rowland I. Antipathogenic activity of probiotics against *Salmonella typhimurium* and *Clostridium difficile* in anaerobic batch culture systems: Is it due to synergies in probiotic mixtures or the specificity of single strains. Anaerobe. 2013; 24:60-65. doi: 10.1016/j.anaerobe.2013.09.011

37. El-Kholy AM, El-Shinawy SH, Meshref AMS, Korny AM. Screening of antagonistic activity of probiotic bacteria against some food-borne pathogens. J Food Biosci Technol. 2014; 4: 1-14. doi: 10.12691/jaem-2-2-4

38. Rossland E, Borge GI, Langsrud T, Sorhaug T. Inhibition of *Bacillus cereus* by strains of Lactobacillus and Lactococcus in milk. Int J Food Microbiol. 2003; 89(2-3): 205-212. doi. 10.1016/S0168-1605 (03)00149-1

39. Prudencio CV, Dos Santos MT, Vanetti MC. Strategies for the use of bacteriocins in Gram-negative bacteria: Relevance in food microbiology. J Food Sci Technol. 2015; 52(9):5408-4017. doi: 10.1007/s13197-014-1666-2

http://dx.doi.org/10.22037/afb.v5i3.21127

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2018, 5 (3): 173-183
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

استفاده از *لاکتوباسیلوس اسیدوفیلوس* و *بیفیدوباکتریوم انیمالیس زیر گونه لاکتیس ب ب ۱۲*، به عنوان زیست یار به منظور کاهش خطر مسمومیت غذایی ناشی از گوشت چرخ کرده

جواد کاظم عیسی[۱]، سید هادی رضوی[۲*]

۱- گروه علوم و مهندسی صنایع غذایی، دانشکده مهندسی و فناوری کشاورزی، دانشگاه تهران، کرج، ایران.

۲- قطب علمی کاربرد فناوری های نوین به منظور تولید نوشیدنی ها و مواد غذایی فراسودمند و آزمایشگاه مهندسی زیست فرآیند (BPEL)، گروه علوم و مهندسی صنایع غذایی، دانشکده مهندسی و فناوری کشاورزی، دانشگاه تهران، کرج، ایران.

چکیده

سابقه و هدف: برای اولین بار متابولیت‌های ضدمیکروبی تولید شده توسط زیست‌یارها به عنوان جایگزین طبیعی نگهدارنده‌های شیمیایی در جهت کمک به سلامت مصرف کننده انجام شد. این مطالعه از باکتری های *لاکتوباسیلوس اسیدوفیلوس* و *بیفیدو باکتریوم انیمالیس زیر گونه لاکتیس* به عنوان کشت‌های محافظ و ترکیبات ضدمیکروبی آنها برای کاهش خطر مسمومیت غذایی ناشی از گوشت چرخ کرده استفاده شد.

مواد و روش‌ها: نمونه های گوشت چرخ شده در دمای ۴ درجه سلسیوس نگهداری شدند. آزمون میکروبی زیست‌یارها و بیماری‌زاها در روزهای ۰، ۳، ۷ و ۱۴ انجام شد. در این دوره زمانی، pH و فعالیت ضدمیکروبی زیست‌یارها مورد بررسی قرار گرفت.

یافته‌ها و نتیجه‌گیری: طی زمان نگهداری در سرما تعداد میکروب‌های بیماریزای تلقیح شده به نمونه‌های گوشت چرخ کرده حاوی هر یک از زیست‌یارها، در سطوح گوناگون کشت همزمان با باکتری‌های پروبیوتیک به طور معنی‌دار ($p\leq۰/۰۵$) و غیرمعنی‌دار ($p\geq۰/۰۵$) کاهش یافت. زیست‌یارها نشان دادند که قابلیت تولید ترکیبات ضدمیکروبی (اسید لاکتیک، دی استیل، پراکسید هیدروژن) در غلظت‌های گوناگون را دارند که تا پایان دوره نگهداری نمونه‌های گوشت چرخ کرده در سردخانه به طور معنی‌داری این میزان افزایش می‌یابد ($p\leq۰/۰۵$). در بررسی نشان داده شد که زیست‌یارها قابلیت تولید باکتریوسین‌ها را دارند. *لاکتوباسیلوس اسیدوفیلوس* به عنوان یک زیست‌یار اثر معنی‌داری به عنوان یک نگهدارنده طبیعی در برابر میکروب های بیماری‌زا داشت و تأثیر آن هنگام ترکیب با *بیفیدو باکتریوم ب ب ۱۲* به مقدار چشمگیری افزایش یافت.

تعارض منافع: نویسندگان اعلام می کنند که هیچ تعارض منافعی وجود ندارد.

تاریخچه مقاله

دریافت ۲۱ آپریل ۲۰۱۸
داوری ۱۱ ژوئن ۲۰۱۸
پذیرش ۲۳ ژوئن ۲۰۱۸

واژگان کلیدی

- ترکیبات ضدمیکروبی
- باکتریوسین
- نگهدارنده طبیعی
- نگهداری سرد
- باکتری های بیماری زا

***نویسنده مسئول**

سید هادی رضوی، گروه علوم و مهندسی صنایع غذایی، دانشکده مهندسی و فناوری کشاورزی، دانشگاه تهران، کرج، ایران.

تلفن: ۰۹۸-۲۶۳-۲۲۴۸۸۰۴+
دورنگار: ۰۹۸-۲۶۳-۲۲۴۹۴۵۳+
پست الکترونیک:
srazavi@ut.ac.ir