The combined effect of bacteriocin-producing *Lactobacillus rhamnosus* GG-ATCC and Lactoperoxidase system activation on microbiological quality of raw milk with special emphasis against *E.coli* O157:H7 in milk

Najim Hadi Najim and Farah Ibrahim Mustafa
Department of Veterinary Public Health, College of Veterinary Medicine, Baghdad University, Iraq.
E-mail: nhnh52@yahoo.com
Received: 11/11/2015; Accepted: 12/1/2016

Summary

Isolates of Enterohaemorrhagic *E.coli* O157:H7 were isolated from 51 and 41 of locally produced bovine and ovine soft cheese samples. Their identification were confirmed based on the biochemical reactions and both the morphological cultural and serological properties. Presumptive *E.coli* O157:H7 isolates obtained by using the conventional selective plating on the chromogenic agar were tested further for the presence of both O157 and H7 antigenes using the latex agglutination test antisera. The current microbiological studies revealed that 31 (33.70 %) out of 92 bovine and ovine soft cheese samples were positive for *E.coli* O157:H7. The highest non significant (P>0.05) prevalence level of *E.coli* O157:H7 was found in the ewe's soft cheese samples (36.59 %) followed by cow's soft cheese samples (31.37 %). Agar well diffusion bioassay method was used for measuring the antibacterial activity of the crude bacteriocin that was produced by *Lactobacillus rhamnosus* GG-ATCC against *Escherichia coli* and the closely related sensitive strains such as *L.acidophilus* LA-K and *L.acidophilus* ROO52. The crude bacteriocin that was produced by the *L.rhamnosus* GG-TACC exhibited significantly (P<0.05) the highest antibacterial potency (100 %) against both the closely related strains of lactobacilli and the stressed *E.coli* O157:H7 by the activation of the LPS. The activation of the natural LPS of inoculated pasteurized milk had significantly (P<0.05) influenced the inactivation degree of the crude bacteriocin against *E.coli* O157:H7. There was a significant (P<0.05) reduction in the viable counts of stressed *E.coli* O157:H7 after each exposure time period (6, 24 and 48 hrs.) to the crude bacteriocin at room storage temperature. An overall conclusion on the basis of the current results pointed out that complete elimination of viable bacterial cells was not achieved neither in the stabilized milk (Activation of LPS) nor after subjecting the stabilized milk to the action of the crude bacteriocin produced by *L.rhamnosus* GG-ATCC at room storage temperature.

Keywords: Enterohaemorrhagic *E.coli* O157:H7, Raw milk, *Lactobacillus rhamnosus* GG-ATCC, Lactoperoxidase.

Introduction

Milk is a perishable food which is highly susceptible to bacterial spoilage since it can be regarded as a good medium for the growth and multiplication of different kinds of spoilage and pathogenic bacteria (1 and 2). Milk provides all the essential nutrients with a suitable physical environment to the bacteria. In Iraq milk is produced by a large of small holders with small quantities of milk leading to both the milk collection and delivery to the dairy processing plant time consuming. The time delay from milking to delivery was exceeding to six hours which negatively affect the quality of non refrigerated milk (3). Tokar and Teger (4) mentioned that the cattle were a natural reservoir for the shiga toxin producing *E.coli* (STEC) espicially the serotype O157 that could be found in their feces and approximately 75% of the haemorrhagic colitis outbreaks were linked to the consumption of bovine derived products such as raw milk that could be contaminated with such organism through the milking process. Cattle were the major reservoirs and source of human infection which harbored the *E.coli* O157:H7 in their intestinal tracts without showing any clinical symptoms (5 and 6). This condition forced the milk producers to detect simple method for raw milk preservation which could not pose health hazard to the consumers (7). Crude bacteriocin that extracted from *Lactobacillus rhamnosus* GG-ATCC in combination with activation of the naturally

lactoperoxidase system provide new opportunities for controlling the pathogenic *E.coli* O157:H7 and extending milk shelf life (8). This study aimed to inactivate the vital metabolic bacterial enzymes with blocking their metabolism and ability to multiply by the synergistic antibacterial effect of the crude bacteriocin that extracted from *L.rhamnosus* GGATCC in combination with the activated LPS against *E.coli* O157:H7 in raw milk that stored at room temperature (30°C).

Materials and Methods

Isolates of Enterohaemorrhagic *E.coli* O157:H7 were isolated from 51 and 41 of locally produced bovine and ovine soft cheese samples. Their identification were confirmed based on the biochemical reactions and both the morphological cultural and serological properties. Presumptive *E.coli* O157:H7 isolates that obtained by using the conventional selective plating on the chromogenic agar were tested further for the presence of both O157 and H7 antigenes using the latex agglutination test antisera. The crude bacteriocins were obtained from the *Lactobacillus rhamnosus* GG-ATCC, *Lactobacillus acidophilus* LA-K, and *Lactobacillus acidophilus* ROO52, These strains were cultured in MRS broth under anaerobic conditions at 37 °C for 24 hrs. and the supernatant fluid was separated from bacterial cells by centrifugation at 5000 rpm for 30 minutes. The supernatant was collected and its PH was adjusted to 7.0 by using sterile 1N NaOH so as to rule out the inhibition caused by the production of organic acids (lactic acid and acetic acids). The supernatant then was filtered through a syringe filter with pore size of 0.45 µm, then heated at 70°C for 5 min. to prevent inactivation of antibacterial peptides by protease and to kill the bacterial cells and then stored at refrigeration temperature 4°C. Agar well diffusion bioassay method was used for measuring the antibacterial activity of the crude bacteriocin that was produced by *Lactobacillus rhamnosus* GG-ATCC against *Escherichia coli* and the closely related sensitive *L.acidophilus* LA-K and *L.acidophilus* ROO52. Pasturized whole milk was experimentally inoculated with a fixed initial number of $1x10^6$ cfu /ml of *E.coli* O157:H7 and then subjected to the action of the crude bacteriocin after the activation of its natural lactoperoxidase system by using pour plating method on VRB agar and also using a well diffusion bioassy method.

Results and Discussion

The current microbiological studies revealed that 31(33.70 %) out of 92 bovine and ovine soft cheese samples were positive for *E.coli* O157:H7. The highest non significant P>0.05 prevalence level of *E.coli* O157:H7 was found in the ewe's soft cheese samples (36.59%) followed by cow's soft cheese samples (31.37%). The data revealed that there was non-significant P>0.05 prevalence level of *E.coli* O157:H7 isolation between the above mentioned two types of soft cheese samples from AL-Karkh district. (Table, 1).

Table, 1: The prevalence of *E.coli* O157:H7 in bovine and ovine soft cheese samples that were collected from AL-Karkh district of Baghdad city.

Source of cheese samples	No. of samples examined	No. of positive samples	Positive samples %
Cows	51	16	31.37
Sheep	41	15	36.59
Total	92	31	33.70

X_2=0.3 Non significant differences between sheep's and cow's soft cheese.

One gram positive bacterial strain including *L.acidophilus* LA-K and one gram negative bacterial strain including *E.coli* O157:H7 were screened by agar well diffusion assay method for the antimicrobial potency of the crude bacteriocin that produced by the *L.rhamnosus* GGA-TCC. The antimicrobial spectrum exhibited by the crude bacteriocin that produced by the *L.rhamnosus* GG-ATCC against the sensitive strain of *L.acidophilus* LA-K and both the non stressed *E.coli* and the *E.coli* O157:H7 that subjected to a stress action through the activation of milk's lactoperoxidase system is shown in (Table, 2). The average diameter of the clear inhibition zone of the crude bacteriocine that produced by the *L.rhamnosus* GG-ATCC against the closely related strain of *L.acidophilus* LA-K was 16 mm. The test organisms *Escherichia coli* and *E.coli* O157:H7 were resistant to the

crude bacteriocin that was produced by the *L.rhamnosus* GGATCC and absence of clear inhibition zone was detected for each of them after their treatment with the crude bacteriocin and such results were in agreement with (9 and 10).

Table, 2: The antimicrobial spectrum of the crude bacteriocin produced by *Lactobacillus rhamnosus* GG-ATCC against *L.acidophilus* LA-K, *E.coli, E.coli* O157:H7 and stressed *E.coli* O157:H7 by agar well diffusion assay.

Crude bacteriocin of lactobacillus	Sensitive organism	Bacteriocin Activity	Inhibition zone diameter (mm)	Potency %
L.rhamnosus GG-ATCC	*L.acidophilus* LA-K	(+) ve	16	100
L.rhamnosus GG-ATCC	*E.coli*	(-) ve	0	0
L.rhamnosus GG-ATCC	*E.coli* O157:H7	(-) ve	0	0
L.rhamnosus GG-ATCC	Stressed *E.coli* O157:H7 by LPS	(+) ve	16	100

X_2=80 significant differences among types of bacteria, (-) ve: Negative result (no inhibition zone), (+) ve: Positive result (with inhibition zone).

The crude bacteriocin that was produced by *L.rhamnosus* GG-ATCC against *E.coli* O157: H7 that subjected to a stress condition through the activation of the natural lactoperoxidase system in milk was 16 mm. The crude bacteriocin that was produced by the *L. rhamnosus* GG-ATCC exhibited significantly P<0.05 the highest antimicrobial potency (100%) against both the closely related strain of *L.acidophilus* LA-K and stressed *E.coli* O157:H7 by the activation of the LPS while exhibited significantly P<0.05 the lowest antimicrobial potency (0%) against both *Escherichia coli* and non stressed *E.coli* O157:H7 which were exhibited resistance to the crude bacteriocin that was produced by *L.rhamnosus* GG-ATCC where no clear inhibition zone was detected after their treatment with the crude bacteriocin. Activation of the natural lactoperoxidase system of raw milk had significantly P<0.05 influenced the antimicrobial potency of the crude bacteriocin against *E.coli* O157:H7. When the outer membrane of the gram negative bacterial cells was injured by the activation of the natural lactoperoxidase system, the permeation of the crude bacteriocins to the cytoplasmic membrane was facilitated (11). Kieckens (12) reported that activation of the natural LPS distrupted the permeability of the *E.coli* outer membrane and causing periplastic leakage and sensitization to Nisin.

The average mean log values (log cfu/ml) of total aerobic bacterial counts in raw milk samples that were stabilized by the activation of their natural LPS and subjected to the action of the crude bacteriocin that produced by *L.rhamnosus* GG-ATCC and stored at room temperature (30^{o}C) over the four time periods of 0, 6, 24 and 48 hrs. are shown in (Table, 3). Activation of the LPS produced a significant P<0.05 reduction of the total aerobic bacterial counts, where the average mean log value of the starting initial bacterial counts in raw milk was significantly P<0.05 decreased from 6.98±0.108 (96×10^5 cfu /ml) at 0 hr. to 6.36±0.121 (23×10^5 cfu/ml), 5.98±0.105 (97×10^4 cfu/ml) and 5.30±0.102 (25×10^4 cfu/ml) after 6, 24 and 48 hrs. of room storage temperature respectively. The current data revealed that significant P<0.05 differences in the average mean log values of total aerobic bacterial counts were found between the control stabilized raw milk samples and those that subjected to the action of the crude bacteriocin over the time periods of 0, 24 and 48 hrs. but non-significant P>0.05 at 6 hrs. of milk storage at room temperature. There was a significant P<0.05 reduction in the total numbers of aerobic bacterial cells survivors after each exposure time from hours 0 to 48 of milk storage at room temperature. The average mean log value of the starting initial bacterial counts in the control activated raw milk samples decreased significantly P<0.05 from 6.98±0.180 (96×10^5 cfu/ml) to 6.39±0.075 (38×10^5 cfu/ml) immediately after exposure to the crude bacteriocin at 0 hr. and to 6.17±0.038 (15×10^5 cfu/ml), 5.38±0.074 (44×10^4 cfu/ml) and 4.68±0.011 (50×10^3 cfu/ml) after 6, 24 and 48 hrs. of exposure to the action of the crude bacteriocin at room storage temperature respectively.

Table, 3: Effect of crude bacteriocin produced by L.rhamnosus GG-ATCC in combination with activated Lactoperoxidase system (LPS) on the total aerobic bacterial counts (cuf/ml) in raw milk at about 2-3 hours after morning milking then stored at room temperature (30°C).

Storage period (Hours)	Total bacterial counts (log cfu/ ml)	
	Activation of LPS (Control) Mean±SE	Activation of LPS+Bacteriocin Mean±SE
0 hr.	6.98±0.180 96×10^5 Aa	6.39±0.075 38×10^5 Ab
6 hrs.	6.36±0.121 23×10^5 Ba	6.17±0.038 15×10^5 Ba
24 hrs.	5.98±0.105 97×10^4 Ba	5.38±0.074 44×10^4 Cb
48 hrs.	5.30±0.102 25×10^4 Ca	4.68±0.011 50×10^3 Db

LSD=0.6, Different capital letters in a column revealed significant differences (P<0.05) between hours of storage period. Horizontal different small letters revealed significant differences (P<0.05) between the mean values of total aerobic bacterial counts, S.E: Standared Erorr.

The average mean log values (log cfu/ml) of total coliform counts in raw whole milk samples that were stabilized by the activation of their natural LPS and subjected to the action of the crude bacteriocin that produced by the *L.rhamnosus* GG-ATCC and stored at room temperature (30°C) over the four time periods of 0, 6, 24 and 48 hrs. are shown in (Table, 4). Under the conditions used, activation of the natural LPS produced a significant P<0.05 decrease in the total coliform counts with time of milk storage at room temperature, where the average mean log value of the starting initial coliform counts in raw whole milk was significantly P<0.05 decreased from 5.76±0.169 (81×10^4 cfu/ml) at 0 hr. to 5.43±0.111 (31×10^4 cfu/ml), 4.88±0.098 (83×10^3 cfu/ml) and 4.59±0.107 (45×10^3 cfu/ml) after 6, 24 and 48 hrs. of room storage temperature respectively. The time of exposure of stabilized raw milk samples to the action of the crude bacteriocin at room storage temperature had a significant P<0.05 influence on the counts of total coliform bacterial cells survivors from hours 0 to 48. There was a significant P<0.05 decrease in the numbers of coliform bacterial cells survivors after each exposure time period from hours 0 to 48 of milk storage at room temperature. The average mean log value of the starting initial coliform counts in the control activated raw milk samples decreased significantly P<0.05 from 5.76±0.169 (81×10^4 cfu/ml) to 5.12±0.135 (16×10^4 cfu/ml) immediately after exposure to the action of the crude bacteriocin at 0 hr. and to 4.73±0.134 (59×10^3 cfu/ml), 4.32±0.053 (22×10^3 cfu/ml) and to 3.78±0.125 (7×10^3 cfu/ml) after 6, 24 and 48 hrs. of exposure to the action of crude bacteriocin at room storage temperature respectively. The activated raw milk samples that subjected to the action of the crude bacteriocin exhibited significantly P<0.05 the highest antimicrobial effectiveness against the viable coliform bacterial cells in raw milk samples after 48 hrs. of room storage temperature.

Table, 4: Effect of crude bacteriocin produced by L.rhamnosus GG-ATCC in combination with activated lactoperoxidase system (LPS) on the total coliform counts (cfu/ml) in raw milk at about 2-3 hours after morning milking then stored at room temperature (30°C).

Storage Period (Hours)	Coliform counts (log cfu/ml)	
	Activation of LPS (Control) Mean±SE	Activation of LPS+Bactriocin Mean±SE
0 hr.	5.76±0.169 81×10^4 Aa	5.12±0.135 16×10^4 Ab
6 hrs.	5.43±0.111 31×10^4 Ba	4.73±0.134 59×10^3 Bb
24 hrs.	4.88±0.098 83×10^3 Ca	4.32±0.053 22×10^3 Cb
48 hrs.	4.59±0.107 45×10^3 Da	3.78±0.125 7×10^3 Db

LSD=0.3, Different capital letters in a column revealed significant differences (P<0.05) between hours of storage period. Horizontal different small letters revealed significant differences (P<0.05) between the mean values of coliform counts, S.E: Standared Erorr.

The average mean log values (log cfu/ml) of total psychrotrophic bacterial counts in raw whole milk samples that were stabilized by the activation of their natural LPS and subjected to the action of the crude bacteriocin that produced by *L.rhamnosus* GG-ATCC and stored at room temperature over the

periods of 0, 6, 24 and 48 hrs. are shown in (Table, 5). The psychrotrophic viability loss was enhanced by the activation of the LPS with the storage time. The average mean log value of the starting initial psychrotrophic counts in raw whole milk was significantly P<0.05 decreased from 5.69±0.104 (55×10^4 cfu/ml) at 0 hr. to 5.57±0.038 (38×10^4 cfu/ml), 5.35±0.070 (24×10^4 cfu/ml) and 5.24±0.054 (18×10^4 cfu/ml) after 6, 24 and 48 hrs. of room storage temperature, respectively. The current data revealed that significant P<0.05 differences in the average mean log values of total psychrotrophic bacterial counts were found between the control stabilized milk samples and those that subjected to the action of the crude bacteriocin over the four times periods of 0, 6, 24 and 48 hrs. of milk storage at room temperature. There was a significant P<0.05 decrease in the numbers of psychrotrophic bacterial cells survivors after each exposure time period from hours 0 to 48 of milk storage at room temperature. The average mean log value of the starting initial psychrotrophic counts in the control activated raw milk samples reduced significantly P<0.05 from 5.69±0.104 (55×10^4 cfu/ml) to 5.26±0.048 (19×10^4 cfu /ml) immediately after exposure to the action of the crude bacteriocin at 0 hr. and to 4.93±0.060 (9×10^4 cfu/ml), 4.56±0.079 (39×10^3 cfu /ml) and 4.12±0.073 (14×10^3 cfu/ml) after 6, 24 and 48 hrs. of exposure to the crude bacteriocin at room storage temperature respectively (Table, 5). The activated raw milk samples that subjected to the action of the crude bacteriocin exhibited significantly P<0.05 the highest antimicrobial effectiveness against the viable psychrotrophic bacterial cells after 48 hrs. of room storage temperature. One liter of raw whole milk that was screened to be free from *E.coli* O157:H7 was experimentally pasteurized at 63°C for 30 min. inside a water bath. The natural LPS was activated in the first portion by treating it with 15 ml of sodium thiocyanate stock solution and 5 ml of hydrogen peroxide stock solution while the natural LPS was not activated in the second portion of pasteurized milk to serve as a control. Both portions of pasteurized milk were experimentally inoculated with a fixed initial number of 1×10^6 cfu/ml of *E.coli* O157:H7. the efficacy the crude bacteriocin that produced by the *L.rhamnosus* GG-ATCC against stressed *E.coli* O157:H7 by activation of LPS in the first portion of pasteurized milk was tested with five replications, while the second portion of inoculated pasteurized milk was not subjected to the action of the crude bacteriocin but the same amount that was used in the first portion was replaced by sterile peptone water (0.1% wt/v) to serve as a control. Both portions of inoculated pasteurized milk were stored at room temperature (30°C) for three time periods of 6, 24 and 48 hrs.

Table, 5: Eeffect of crude bacteriocin produced by *L.rhamnosus* GG-ATCC in combination wih activated Lactoperoxidase system (LPS) on the total psychrotrophic counts (cfu/ml) in raw milk at about 2- 3hours after morning milking then stored at room temperature (30°C).

Storage period (Hours)	Psychrotrophic counts (log cfu/ml)	
	Activation of LPS (Control) Mean±SE	activation LPS +Bacteriocin Mean±SE
0 hr.	5.69±0.104 55×10^4 Aa	5.26±0.048 19×10^4 Ab
6 hrs.	5.57±0.038 38×10^4 Ba	4.93±0.060 9×10^4 Bb
24 hrs.	5.35±0.070 24×10^4 Ca	4.56±0.079 39×10^3 Cb
48 hrs.	5.24±0.054 18×10^4 Ca	4.12±0.073 14×10^3 Db

LSD=0.2, Different capital letters in a column revealed significant differences (P<0.05) between hours of incubation. Horizontal different small letters revealed significant differences (P<0.05) between the mean values of psychrotrophic counts. S.E: Standard Error.

There was a significant P<0.05 increase in the *E.coli* O157:H7 counts over the three time periods of room storage temperature in the control milk samples that were neither stabilized by the activation of their LPS nor subjected to the action of the crude bacteriocin. The average mean log value of the starting initial count of *E.coli* O157:H7 in the control milk samples increased significantly P<0.05 from 5.98±0.050 (10×10^5 cfu/ml) at 0 hr. to 7.28±0.049 (20×10^6 cfu/ml), 7.92 ±0.118 (99×10^6 cfu/ml) and to 8.25±0.068

(18×10^7 cfu/ml) after 6, 24 and 48 hrs. of milk storage at room temperature, respectively (Table, 6). There was a significant $P<0.05$ decrease in the viable counts of stressed *E.coli* O157:H7 after each exposure time period from hours 6 to 48 of milk storage at room temperature, where the average mean log value of an initial count of 5.98±0.050 (10×10^5 cfu/ml) at 0 hr. significantly $P<0.05$ decreased to 5.84±0.020 (70×10^4 cfu/ml), 5.47± 0.082 (30×10^4 cfu /ml) and 4.83 ± 0.049 (70×10^3 cfu/ml) survivor cells of *E.coli* O157:H7 after 6, 24 and 48 hrs. of exposure to the action of the the crude bacteriocin at room storage temperature, respectively.

Table, 6: The antimicrobial spectrum of the crude bacteriocin produced by *L.rhamnosus* GG-ATCC in combination with activated LPS on the survival rate of *E.coli* O157:H7 (cfu/ml) in pasteurized milk stored at room temperature (30°C).

Storage period (Hours)	Counts of *E.coli* O157:H7 (log cfu/ml)	
	Control Mean±SE	Combination of bacteriocin with LPS Mean±SE
0 hr.	5.98±0.050 10×10^5 Da	5.98±0.050 10×10^5 Aa
6 hrs.	7.28±0.049 20×10^6 Ca	5.84±0.020 70×10^4 Bb
24 hrs.	7.92±0.118 99×10^6 Ba	5.47±0.082 30×10^4 Cb
48 hrs.	8.25±0.068 18×10^7 Aa	4.83±0.049 70×10^3 Db

LSD=0.15, Different capital letters in a column revealed significant differences (P<0.05) between hours of incubation. Horizontal different small letters revealed significant differences (P<0.05) between the mean values of *E.coli O157:H7* counts. S.E: Standard Error.

An overall conclusion on the basis of the current results pointed out that complete elimination or inactivation of viable cells of *E.coli* O157:H7 was not achieved after subjecting of stabilized milk to the action of the crude bacteriocin produced by the *L.rhamnosus* GG-ATCC at room storage temperature. Exposure of stabilized milk to the action of the crude bacteriocin resulted in a reduction of viable count of *E.coli* O157:H7 from (10×10^5 cfu/ml) at 0 hr. to (70×10^3 cfu/ml) after 48 hrs. of storage at room temperature. This result was in agreement with (13) who reported that exposure of stabilized milk to the action of the crude bacteriocin produced by the *L.acidophilus* LA-K resulted in reduction of viable count of 3.935 log cfu/ml of *E.coli* O157:H7 after 48 hrs. of milk storage at room temperature.

References

1. Amigo, N.; Mercado, E.; Bentancor, A.; Singh, P.; Vilte, D.; Gerhardt, E.; Zotta, E.; Ibarra, C.; Manning, S. D.; Larzabal, M. and Cataldi, A. (2015). Clade 8 and Clade 6 strains of *E.coli* O157:H7 from cattle in Argentina. PLOS ONE. 10(6):0127710.
2. Barabas, J. (1994). Milk hygiene with practical reference to milk handling and preservation. Proc. regional workshop on Raw Milk Handling Preservation, Univ. Alex. Egypt.
3. Sangoyomi, T. E.; Owoseni, A. A. and Okerokun, O. (2010). African J. Microb. Res., 4(15):1624-1630.
4. Tokar, K. G. and Teger, S. E. (2008). Acta Agri-Sloveniea. 92(1):61-74.
5. Daher, N. A. (2013). The synergistic bacteriocidal effect of bacteriocin and pressurization against *E. coli* O157:H7 in raw milk. M.Sc. Thesis, Collage of Veterinary Medicine, University of Baghdad.
6. Nguyen, Y. and Sperandio, V. (2012). Entero -haemorrhagic *E.coli* (EHEC) pathogenesis. Front. Cell Infect. Microbi., 2:90.
7. Ryoba, R.; Bakuname, M. and Kurwijila, R. (2000). Potential need for alternative milk preservative system in Tanzania. LPS workshop, Dar Essalaam.
8. Khudhier, Z. S. (2011). Antibacterial activity of L. acidophilus bacteriocin against *E. coli* O157:H7 in raw milk. PhD thesis, College of Veterinary Medicine, University of Baghdad.
9. Kalchayanand, N.; Hanlin, M. B. and Ray, B. (1992). Sublethal injury makes gram-negative and resistant gram-positive bacteria sensitive to the bacteriocins, pediocin AcH and nisin. Lett. Appl. Microb., 15:239-243.
10. FSANZ, (2002). Food Standards Australian New Zealand. Application A 404-Lactoperoxidase system. Final Assessment Reporte.

11. Hauben, K.; Wuytack, E.; Soontjens, C. and Michiels, C. (1996). High pressure transient sensitization of Escherichia coli to lysozyme and nisin by disruption of outer-membrane permeability. J. Food Prot., 59: 350-355.

12. Kieckens , E.; Rybarczyk, J.; De Zutter, L.; Duchateau, D.; Vanrompay, D. and Cox, E. (2015). Clearance of *E.coli* O157:H7 infection in calves by Rectal Administration of bovine lactoferrin. Appl. Environ. Microb.; 81(5):1644-1651.

13. Wali, M. K. (2014). The synergistic bactericidal effects of bacteriocin and activated lactoperoxidase system against *E.coli* O157:H7 in raw milk. M.Sc. thesis college of Vet. Medicine, University of Baghdad.

الفعل التآزري للبكتريوسين المنتج من بكتريا *Lactobacillus rhamnosus* ونظام اللاكتوبيروكسيديز المفعل على النوعية الجرثومية للحليب الخام مع التأكيد ضد الاشيريشيا القولونية المعوية النزفية O157:H7 في الحليب

نجم هادي نجم و فرح ابراهيم مصطفى

فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة بغداد، العراق.

E-mail: nhnh52@yahoo.com

الخلاصة

عزلت الايشريشيا القولونية المعوية النزفية O157: H7 من 51 و41 عينة من الجبن الطري المحلي للأبقار والأغنام على التوالي. شخصت عزلات الأيشريشيا القولونية النمط المصلي O157:H7 اعتماداً على تفاعلاتها البايوكيميائيه وخصائصها الزراعية والمصلية. أجريت العديد من الفحوصات المصلية على العزلات الافتراضية للايشريشيا القولونية O157: H7 والتي حُصِلَ عليها باستعمال الوسط الزرعي الانتقائي الصباغي بحثا عن المستضد الجسمي (O157) والمستضد السوطي (H7) باستعمال عدة اختبار تلازن اللاتكس التجاري المتوافرة لهذا النمط. أشارت الدراسة الميكروبية الحالية للعزلات ألزراعية إلى أن 31(33.70%) عينه من اصل 92 عينه من عينات الاجبان الطرية المحلية للأبقار والأغنام كانت موجبه للايشريشيا القولونية المعوية النزفية (O157:H7). وجد مستوى انتشار عالٍ غير معنوي (P>0.05) لتلوث عينات الأجبان الطرية للأغنام بالاشريشيا القولونية (O157:H7) وبنسبه 36.59% يليها عينات الاجبان الطرية للأبقار وبمستوى انتشار 31.37%. استعمل الاختبارالاحيائي للانتشار في الحفر عبر الأكار لتقييم الفعالية التثبيطية للبكتريوسين الخام المنتج من السلألة *Lactobacillus rhamnosus* GG-ATCC ضد الأيشريشيا القولونية والسلالات الحساسة من العصيات اللبنية مثل *L.acidophillus* LA-K و*L.acidophillus* ROO52. اعتبرت النتائج ايجابية في حالة ظهور 2 مليمتر أو أكثرمن مناطق التثبيط حول كل حفرة. وكان معدل قطر منطقه التثبيت للبكتريوسين الخام المنتج من*L.rhamnosus* GG-ATCC ضد كل من *L.acidophilus* L-AK و *L.acidophillus* ROO52 هو 16 مليمترفي حين ان الايشيريشيا القولونية والأيشريشيا ألقولونية النمط المصلي (O157:H7) ابدت مقاومتها للبكتريوسين الخام إذ إنها لم تظهر اي منطقة تثبيط حول اي حفرة عوملت بنفس البكتريوسين الخام. ازدادت حساسية الايشيريشيا القولونية النمط المصلي O157:H7 للبكتريوسين الخام بعد تعرضها للاجهاد من خلال تنشيط نظام اللاكتوبيروكسديز وكان معدل قطر منطقة التنشيط للبكتريوسين الخام المنتج *L.rhamnosus* GG-ATCC ضد الايشريشيا القولونية المجهدة بتنشيط نظام اللاكتوبيروكسديز الطبيعي هو 16 مليمتر. أظهر البكتريوسين الخام المنتج من *L.rhamnosus* GG-ATCC اعلى مستوى للفعالية التثبيطيه 100% بمستوى معنوي (P<0.05) ضد كل من العصيات اللبنية والايشريشيا القولونية النمط المصلي O157:H7 التي تعرضت للإجهاد بتنشيط نظام اللاكتوبيروكسديز الطبيعي في الحليب الخام. كذلك صممت الدراسة لتقييم قوه الفعالية التثبيطيه للبكتريوسين الخام المنتج من *L.rhamnosus* GG-ATCC بالتآزر مع تنشيط نظام اللاكتوبيروكسديز ضد حيوية الايشريشيا القولونية النمط المصلي O157:H7 في الحليب المبسترعبر المدد الزمنية 6 و24 و48 ساعة من حفظ الحليب عند درجه حرارة الغرفة .كان لعمليه تنشيط نظام اللاكتوبيروكسديز في الحليب المبستر تاثيراً معنويا (P<0.05) على قوه البكتريوسين الفعالة لتثبيط نمو بكتريا الايشريشيا القولونية النمط المصلي O157:H7 وفقدان حيويتها. انخفض مستوى الاعداد الكلية للايشريشيا القولونية O157:H7 وبصوره معنوية (P<0.05) خلال 6 و24 و48 ساعة من تعرض الحليب المبستر لفعل البكتريوسين الخام بالتآزر مع تفعيل نظام اللاكتوبيروكسديز عند درجه حرارة الغرفة. تستنتج الدراسة إنه لم يتحقق القضاء الكامل لخلايا البكتريا الحيه من خلال تنشيط نظام اللاكتوبيروكسديز فقط ألا من الفعل التازري للبكتريوسين مع تنشيط نظام اللاكتوبيروكسديز عند حفظ الحليب بدرجه حراره الغرفة.

الكلمات المفتاحية: الاشيريشيا القولونية المعوية النزفية O157:H7، الحليب الخام، نظام اللاكتوبيروكسيديز المفعل.