The synergistic bactericidal effects of bacteriocin and pressurization against *E.coli* O157:H7 in raw milk

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Summary

Colonies of *E.coli* O157:H7 were isolated from 35 raw milk sample and their identification were confirmed based on biochemical reactions and both cultural and serological characteristics. Presumptive *E.coli* O157:H7 isolates obtained by selective plating on both CT-SMAC and Chromogenic agars were further tested serologically for the presence of both O157 and H7 antigenes using the commercial available latex agglutination test kit. The unhygienic practices in the production of milk in Al-Thahab Al- Abiedh , Abu-Graib, Al-Zedan and Khan Dharie were reflected on the highest significant ($p<0.01$)prevalence level of contamination with *E.coli* O157:H7 that appeared to be 80%, 80%, 60% and60% respectively. Homogenization pressure of 1000 psi and 2000 psi for five passes had significantly ($p<0.05$) influenced the inactivation degree of *E.coli* O157:H7 in both whole milk and nutrient broth. Milk homogenized at a pressure level of 3000 psi for three passes and 4000 psi for two passes resulted in a further increase of the antimicrobial effectiveness and produced an additional significant ($p<0.05$) reduction of *E.coli* O157:H7. Complete elimination (inactivation) of viable *E.coli* O157:H7 was achieved when cultured whole milk was homogenized at pressure level of 5000 psi for a single pass. Agar well diffusion bioassay was used for the evaluation of antimicrobial activity of the crude bacteriocin produced by *L.acidophilus* LA-K against *E.coli* O157:H7. Enterohaemorrhagic *E.coli* O157:H7 expressed its resistance to the crude bacteriocin since it did not show any inhibition zone around each well treated with bacteriocin. The average diameters of the inhibition zones of crude bacteriocin against stressed *E.coli* O157:H7 by pressurization at 4000 psi, 3000 psi, 2000 psi and 1000 psi were 14 mm, 12mm, 10mm and 8mm respectively. The homogenization pressure level (moderate or high) had significantly ($p<0.05$) influenced the inactivation degree of the crude bacteriocin against the stressed *E.coli* O157:H7 by pressurization. Quantitative measurement of crude bacteriocin antimicrobial activity was determined by using photometric or turbidometric method. The results revealed that no growth of stressed *E.coli* O157:H7 with no visible turbidity in the nutrient broth with bacteriocin that diluted to 1/2, 1/4 and 1/8 were observed. Bacteriocin that diluted to 1/8 which resulted in no visible turbidity after overnight of incubation at 37C° and gave an optical density reading of 1.448.

Keywords: Synergistic Bactericidal, Bacteriocin, Pressurization, E.Coli O157:H7, Raw Milk.

Introduction

Milk is a good medium for the growth of many micro-organisms, since it contains all the necessary nutrients and provides a suitable physical environment; it is therefore a perishable food, highly susceptible to microbial spoilage (1). Typical illness as a result of an *E. coli* O157:H7 infection can be life threatening, and susceptible individuals showed a range of symptoms including hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura (2). Sporadic cases and outbreaks of human diseases caused by *E. coli* O157 have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water (3). As a promising alternative to the heat treatments, homogenization is a fluid mechanical process that involves the subdivision of particles or droplets into micron sizes, to create a stable dispersion or emulsion (4). High pressure transiently disrupts the permeability of the *E.*coli outer membrane for water –soluble proteins (5). Sublethal injury made bacteria more sensitive to other inhibitory factors (6).

Application of natural antimicrobial substances (such as bacteriocins) combined

with novel technologies provides new opportunities for the control of pathogenic bacteria, improving food safety and quality (7). This synergetic inactivation was not only observed in bacteria that were intrinsically sensitive to these peptides (many gram-positive bacteria), but also in gram- negative bacteria, which were normally insensitive because their cellular targets were shielded by an outer membrane (8).

Materials and Methods

E.coli O157:H7 was isolated from 35 raw milk samples after 24 hours of aerobic incubation at 37 C° on both cefixime Tellurite Sorbitol MacConky agar (CT-SMAC) and Chromogenic agar. The identification of *E.coli* O157:H7 was based on cultural, biochemical and serological properties. Presumptive *E.coli* O157:H7 isolates obtained were further tested serologically for the presence of both O157 and H7 antigenes using the commercial latex agglutination test kit.

The crude bacteriocin was obtained from the bacteriocin producing strain *lactobacillus acidophilus* LA-K which was grown in de man Regosa sharp (MRS) broth under anaerobic condition at 37 ^{o}C for 24 hours and the supernatant fluid was separated from cells by centrifugation at 5000 rpm for 30 minutes. The supernatant fluid was collected and the pH was adjusted to 7 with sterile in NaOH so as to rule out inhibition through production of organic acids and filtered through a syringe filter with pore size of 0.45 µm, then heating for 10 minutes at 70 ^{o}C to prevent inactivation of antibacterial peptides by protease and killed all cells and then stored at 4 ^{o}C in a refrigerator. Inhibitory activity of crude bacteriocin against sensitive strain was assayed according to the Method of food microbiology protocols (2001) with slight modification according to (9).

The antimicrobial activity of bacteriocin against indicator organism (*E. coli* O157:H7) was determined using a well diffusion assay (10) after subjecting *E.coli* O157:H7 to a stress condition by the different homogenization pressures (1000psi, 2000psi, 3000psi, 4000psi and, 5000psi). Five identified colonies of *E. coli* O157:H7 by latex agglutinin test kit were selected and subcultured on nutrient agar to obtain pure colonies by incubating at 37 ^{o}C aerobically overnight and then five colonies were inoculated directly in 10 ml of sterile nutrient broth. The inoculated nutrient broth was incubated aerobically at 37 ^{o}C for 24 hours. One ml of the inoculated nutrient broth was serially diluted (10^{-5} to 10^{-6}) in sterile 0.1% (wt/v) peptone water as a diluent and then pour plated. The *E. coli* O157: H7 was enumerated using Violet Red Bile agar VRB agar. The Petri dishes were incubated at 37 ^{o}C for 24 hours and the colonies were counted after the incubation period. Three liters of sterile nutrient broth and three liters of sterile whole milk were inoculated with a fixed number of *E. coli* O157 : H7 of approximately 1 x 10^{6} cfu/ml and then subjected to a stress condition by using different homogenization pressures. The antimicrobial activity of crude bacteriocin produced by the *Lactobacillus acidophilus* LA-K was tested on the growth rate survival of stressed *E. coli* O157: H7 using pour plating method on VRB agar and also by using a well diffusion assay method.

Determination the minimum inhibitory concentration of *Lactobacillus acidophilus* LA-K bacteriocin against stressed *E. coli* O157: H7 was considered as the lowest concentration of the substance to be tested which results in no visible turbidity due to bacterial growth after 24 hours of incubation (11). Growth was measured by determining its turbidity in terms of OD (optical density) at 600 nm by spectrophotometer according to the manufacturing instructions (Optima sp-300, Japan). Effect of bacteriocin on stressed *E. coli* O157 : H7 by pressurization 2000 psi (homogenization pressure)in a liquid medium was determined by growth inhibition of indicator organism at various dilutions of crude bacteriocin which were added to stressed *E. coli* O157 : H7 (10^{6} cfu/ml) in nutrient broth and incubated overnight at 37 ^{o}C . *E. coli* O157: H7 cells without bacteriocin were used as an experimental control (12). Besides that the indicator strain *E. coli* O157: H7 inoculated in nutrient broth with various dilutions of crude bacteriocin were streaked on the nutrient agar and incubated aerobically for overnight at 37 ^{o}C.

Results and Discussion

Typical colonies of *E.coli* O157:H7 appeared on selective enrichment CT-SMC agar as colorless with gray smoky center (13) while on chromogenic agar appeared with typical mauve color (14). The presumptive *E.coli* O157:H7 isolates were motile and unable to grow in the potassium cyanide broth and tested serologically for both O157 and H7 antigens by the commercial latex agglutination kits or antisera. The laboratory studies of the cultural isolation during the period of the study revealed that there was a significant ($p<0.01$) differences in the average viable counts and percentages of *E.coli* O157:H7 isolation between the seven different villages as shown in table, 1 where the highest prevalence level of *E.coli* O157:H7 were found in both Al-thahab Al-Abiedh and Abu-Graib (80%) followed by Al-Zedan and Khan-Dharie (60%), followed by Al-Radhwania (40%) and finally followed by the animal fields of both the Agricultural and the Veterinary Colleges (20%). Out of 35 raw milk samples examined only 18 (51.54%) samples were positive for *E.coli* O157:H7 (Table 1), and such high prevalence level of contamination with *E.coli* O157:H7 pointed out the potential public health hazared. The faeces may contaminate the udder and milking equipments and get into milk during milking and handling if adequate hygienic practices are not observed (15).

Table, 1: The prevalence (%) and count of *E.coli* O157:H7in raw milk samples from different villages surrounding Baghdad province.

location	No. of samples	No. and (%)of (+) ve samples	Mean count±SE of *E.coli* O157:H7 log_{10} cfu/ml
Al-Thahab Al- Abiedh	5	4 (80%) A	4.38 ± 0.08
Abu-Graib	5	4 (80%) A	4.146 ± 0.06
Al-Zedan	5	3 (60%) B	3.146 ± 0.04
Khan-Dharie	5	3 (60%) B	3.00 ± 0.04
Al-Radhwania	5	2 (40%) C	3.00 ± 0.04
Animal Field of (Agri. College)	5	1 (20%) D	2.78 ± 0.00
Animal Field of (Vet. College)	5	1 (20%) D	2.30 ± 0.00
Total	35	18 (51.54%)	3.77
LSD value		8.55**	0.439**
p-value		0.0017	0.00068

Different letters reveled significant differences (P<0.01).

Effect of different homogenization pressures on the viability loss of *E. coli* O157:H7 in milk and nutrient broth at level (1000 psi and 2000 psi) for five passes had significantly ($P<0.05$) influenced the inactivation degree of *E. coli* O157:H7 in both the whole milk and nutrient broth. As the homogenization pressure increased from 1000 psi to 2000 psi, the inactivation of *E. coli* O157:H7 population increased and resulted in a decrease of viable count of 0.426 log cfu/ml in milk and 0.701 log cfu/ml in nutrient broth. Besides that this study demonstrates that the homogenization pressure was more effective against *E. coli* O157:H7 in nutrient broth than in whole milk because the reduction in the viable counts of *E. coli* O157:H7 was significantly ($P < 0.05$) lower in whole milk than that obtained in nutrient broth (Table, 2). This difference may be attributed to differences in the composition of the media (16). Among the various milk constituents, fat would most likely provide a protective effect for microorganisms against unfavorable conditions (17).

Influence of moderate and high homogenization pressures on the viability loss of *E. coli* O157:H7 in whole milk:

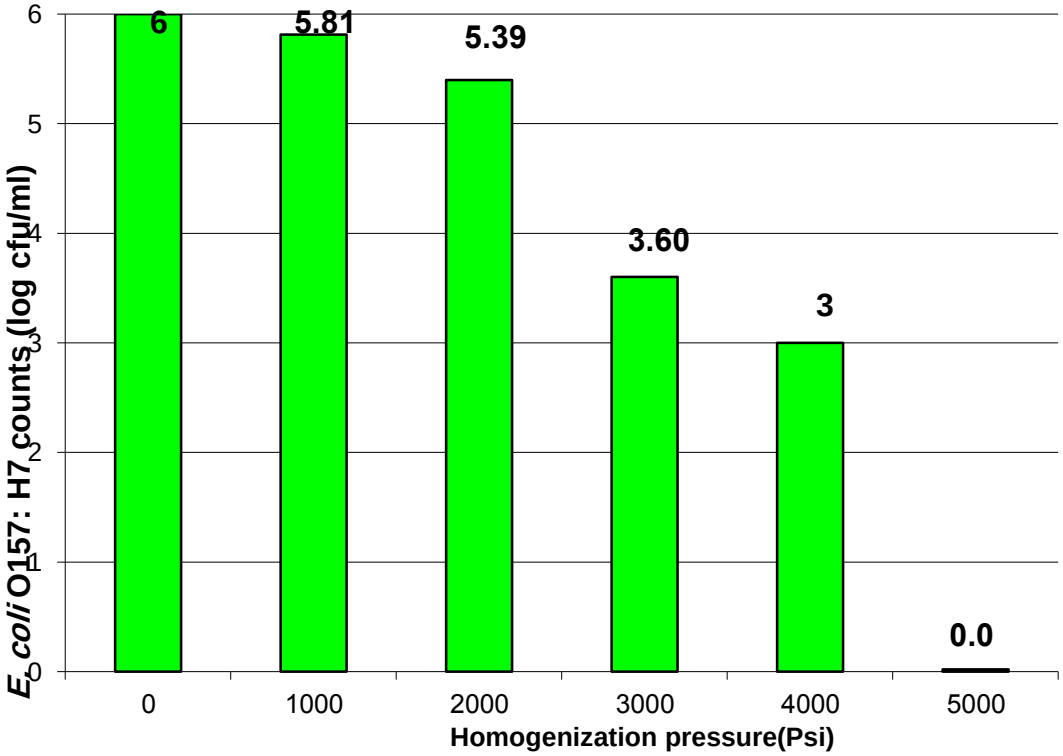
Homogenization pressure level for different number of passes had significantly ($P < 0.05$) influenced the inactivation degree of *E. coli* O157:H7 in whole milk (Figure 1). Under the conditions used, there was no significant ($P > 0.05$) reduction of the starting initial count of 1×10^6 cfu/ml (6 log cfu/ml) at atmospheric pressure (0 psi). Pressure level of 1000 psi for five passes produced a significant ($P < 0.05$) reduction of *E. coli* O157:H7 to 65×10^4

cfu/ml (5.81 log cfu/ml) in milk while increasing the pressure level up to 2000 psi for five passes increased the inactivation of *E. coli* O157:H7 to 25 × 10^4 cfu/ml (5.39 log cfu/ml). Pressure level of 3000 psi for three passes produced an additional significant ($P < 0.05$) reduction of *E. coli* O157:H7 to 4×10^3 cfu/ml (3.60 log cfu/ml) in milk i.e. 2.40 log reduction in cfu/ml , while increasing the increased pressure level and number of passes (18).

pressure level up to 4000 psi for two passes resulted in a further reduction of cell number of *E. coli* O157:H7 to 1 × 10^3 cfu/ml (3 log cfu/ml) i.e. resulted in a decrease of viable count of 3 log cfu/ml. Complete elimination (inactivation) of viable *E. coli* O157:H7 (6 log reduction in cfu/ml) was achieved when whole milk was pressurized at 5000 psi for a single pass. Microbial inactivation increasing with

Table, 2: Effect of different homogenization pressures (1000 and 2000 Psi) for five passes on the viability losses of *E. coli* O157: H7 in milk and nutrient both.

Type	Counts of E. coli O157: H7 (log cfu/ ml)			LSD value
	Before homogenization	After homogenization		
		1000 Psi	2000 Psi	
Whole milk	6.778	6.602	6.176	0.248 *
Nutrient broth	6.778	6.301	5.600	0.407 *
LSD value	----	0.216 *	0.353 *	----
* (P<0.05).				



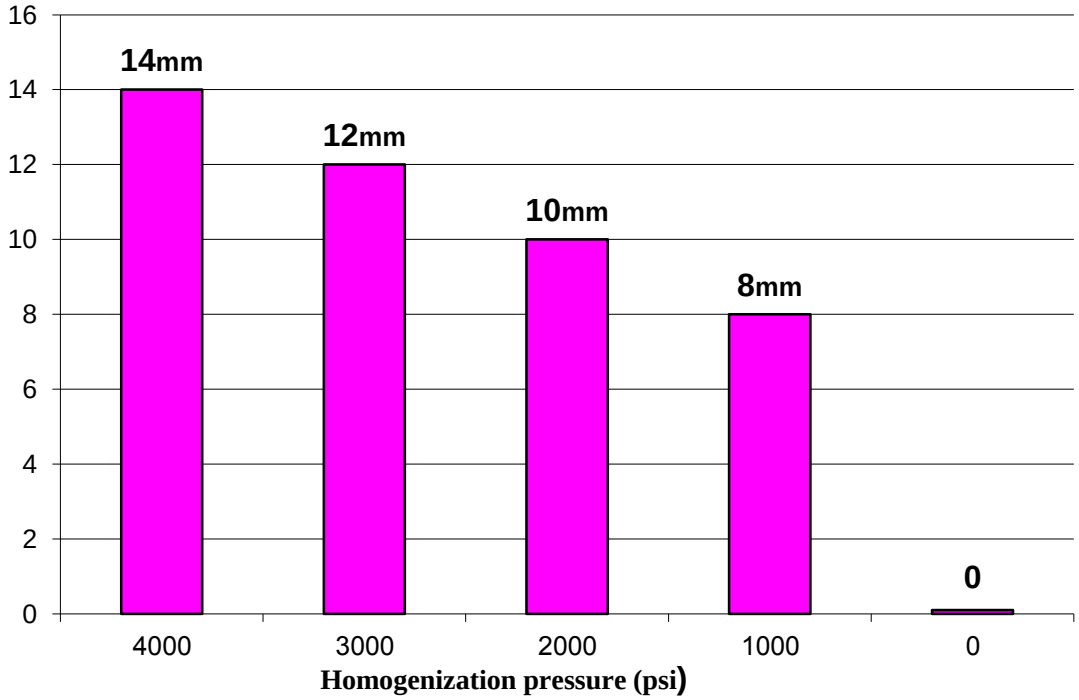
Figure, 1: Influnce of moderate and high hommogenization pressures on the viability loss of *E. coli* O157: H7 in milk.

The antimicrobial spectrum of crude bacteriocin produced by *L. acidophilus* LA-K against stressed *E. coli* O157:H7 by different

pressurization. Sterile whole milk was inoculated with an initial count of 1 x 10^6 cfu/ml of *E. coli* O157:H7 and then subjected

to different homogenization pressures at 1000 psi, 2000 psi , 3000 psi , and 4000 psi .

The antimicrobial activity of the crude bacteriocin obtained from *L. acidophilus* LA-K against stressed *E. coli* O157:H7 by pressurization of milk was evaluated by well diffusion method. The average diameters of the inhibition zones of crude bacteriocin that were produced by *L. acidophilus* LA-K against stressed *E. coli* O157:H7 by pressurization of milk at 4000 psi, 3000 psi, 2000 psi and 1000 psi were 14 mm, 12 mm, 10 mm, and 8 mm respectively (figure 2). The unstressed (0 psi) *E.coli* O157:H7 in milk was resistant to the crude bacteriocin where no clear inhibition zone was detected after its treatment with the bacteriocin. Pressurization of milk had significantly ($P < 0.05$) influenced the antimicrobial activity of bacteriocin against *E. coli* O157:H7. Pressurization inflicted sublethal injury in the cell wall and cell membrane (cell envelope) of gram – positive and gram – negative survivors, which became susceptible to the bacteriocins(19).



Figure, 2: The antimicrobial spectrum of crude bacterocin produced by *L. acidophilus* LA-K against stressed *E. coli* O157: H7 by pressurizatioin.

Effectiveness of crude bacteriocin on the viability loss of stressed *E. coli* O157:H7 by pressurization at 1000 and 2000 psi in nutrient broth and milk:

Three liters of sterile nutrient broth and whole milk were inoculated by *E. coli* O157:H7 with an initial count of 6 x 10^6 cfu/ml and then subjected to homogenization pressure at either 1000 psi or 2000 psi for five passes. The count of survivor cells of stressed *E. coli* O157:H7 by pressurization that subjected to the crude bacteriocin was monitored every 30 minutes for 120 minutes of refrigeration storage using the pour plating method on VRB agar, and the colonies were counted after the aerobic incubation at 37 °C for 24 hours. The homogenization pressure level (1000 psi and 2000 psi) for five passes had significantly ($P < 0.0001$) influenced the inactivation degree of crude bacteriocin against stressed *E. coli* O157:H7 (Table 3 and 4). The time of exposure to the crude bacteriocin at refrigeration storage had a significant ($p<0.0001$) influence on the viability loss of stressed *E.coli* O157:H7 by pressurization from minutes 30 to 120. At the pressurization level of either 1000 psi or 2000 psi for five passes there was a significant ($P < 0.0001$) decrease in the viable counts of stressed *E. coli* O157:H7 that subjected to the

crude bacteriocin after each refrigeration storage time of 30 minutes (Tables 3 and 4). The degree of inactivation of *E.coli* O157:H7 in milk (Table 4) was lower than that in nutrient broth (Table 3) in presence of bacteriocin and this can be attributed to the milk fat and other components such as protein, sugar and mineral salts which played a more important role in the protective effect of milk compared with phosphate buffer saline (PBS) (20). The higher activity of hydrostatic pressure in combination with nisin on the inactivation of *E.coli* was reported in phosphate buffer saline (PBS) (21).

Table, 3: Effectiveness of crude bacteriocin on the viability loss of stressed *E. coli* O157: H7 by pressurization in nutrient broth.

Refrigeration storage time (Minutes)	Log of Count of stressed *E. coli* O157: H7 by pressurization (cfu.ml)	
	1000 Psi	2000 Psi
0: Control	6.00 A	5.40 A
30 Min.	5.30 B	5.30 B
60 Min.	5.20 C	5.18 C
90 Min.	4.95 D	4.70 D
120 Min.	4.85 E	4.38 E
LSD value	0.044	0.029
P-value	0.0001	0.0001

Different letters in column revealed significant differences (P<0.0001) between the refrigeration storage time.

Table, 4: Effectiveness of crude bacteriocin on the viability loss of stressed *E. coli* O157: H7 by pressurization in milk.

Refrigeration storage time (Minutes)	Log of Count of stressed E. coli O157: H7 by pressurization (cfu.ml)	
	1000 Psi	2000 Psi
0: Control	6.30 A	5.88 A
30 Min.	5.46 B	5.48 B
60 Min.	5.36 C	5.30 C
90 Min.	5.08 D	4.70 D
120 Min.	4.95 E	4.48 E
LSD value	0.041	0.053
P-value	0.0001	0.0001

Different letters in column revealed significant differences (P<0.0001) between the refrigeration storage time.

Effectiveness of crude bacteriocin on the viability loss of stressed *E.coli* O157:H7 by subjecting whole milk to different high homogenization pressures.

Bacteriocins and hydrostatic pressure produced cell death by somewhat similar mechanisms, a combination of the two would be more effective in destroying cells of target bacteria, than either of them alone(22). The count of survivor stressed *E.coli* O157:H7 by pressurization that subjected to the crude bacteriocin was monitored every 30 minutes for 120 minutes of refrigeration storage using the pour plating method on VRB agar and the colonies were counted following the aerobic incubation at 37C° for 24 hours (Table 5). Homogenization of milk at pressure level of 3000 psi for five passes produced a significant ($p< 0.05$) reduction in the viability counts of stressed *E.coli* O157:H7 from an initial count of 3.3 log cfu/mlin the control to 3 log cfu/ml survivors after 30 min and to 0 log cfu/ml after 60 minutes of refrigerator storage with the crude bacteriocin. The same trend of viability loss results were obtained when milk homogenized at a pressure level of 4000 psi for two passes which produced a significant ($p< 0.05$) reduction in the viable counts of stressed *E.coli* O157:H7 from an initial count of 2.70 log cfu/ml in the control to only 2 log cfu/ml after 60 min. of refrigeration storage with crude bacteriocin. Complete elimination (inactivation) of viable *E.coli* O157:H7 was achieved when whole milk was pressurized at 5000 psi for a single pass that not subjected to the action of the crude bacteriocin.

Table, 5: Effect of homogenization pressure (3000, 4000, 5000 psi) and bacterocin on viability of *E. coli* O157: H7 in milk.

Homogenization pressure	Counts of *E.coli* O157:H7 (log cfu/ml)					LSD value
	Refrigeration storage time (Min).					
	0 Control	30	60	90	120	
3000 Psi	3.30	3.0	0	0	0	0.520 *
4000 Psi	2.70	2.0	0	0	0	0.318 *
5000 Psi	0	0	0	0	0	0.00 NS
LSD value	0.275 *	0.350 *	0.00 NS	0.00 NS	0.00 NS	

*** (P<0.05), NS: Non-significant.**

Bioassay for quantitative measurements of bacteriocin activity by spectrophotometer the photometric or turbidometric methods have been widely used to offer a simpler, faster and more reliable alternative since the diffusion related problems are eliminated, the degree of human intervention and judgment is low and very low bacteriocin concentrations could be quantified (23). Serial dilutions of crude bacteriocin were made in sterile nutrient broth which was then inoculated with a standardized number (10^6 cfu/ml) of stressed *E. coli* O157:H7 by pressurization (2000 psi for five passes) and incubated aerobically at 37 C^o for overnight. Results of quantitative determination of minimum inhibitory concentration (MIC) by measuring the optical density (OD) at a wave length of 600 nm by optima spectrophotometer are shown in Table (6). Results which are shown in Table (6) revealed that no growth of *E. coli* O157:H7 with no visible turbidity in the nutrient broth was observed with bacteriocin that diluted to 1/2, 1/4 and 1/8 in addition to that no growth was observed by streaking a loop from each of the above mentioned dilutions of the bacteriocin on nutrient agar. The minimum inhibitory concentration (MIC) of the bacteriocin that diluted to 1/8 with optical cell density reading 1.448 was recognized. The growth of *E. coli* O157:H7 with bacteriocin that diluted to 1/16 showed a changes in turbidity with optical cell density reading 1.731 was observed while the growth of the same bacteria in nutrient broth without bacteriocin (as a control) was also observed and gave the optical cell density reading of 2.000 Table (6). Minimum inhibitory concentration (MIC) is often defined simply as the lowest concentration of a substance to be tested at which the turbidity due to bacterial growth was not observed after 24 hours of incubation (24).

Table, 6: Turbidmetric assay for minimum inhibition concentration of the bacteriocin by using spectrophotometer

Tube number	Dilutions Of bacteriocin	O .D. 600 nm Before incubation (mean of replications)	O .D. 600 nm After incubation overnight (mean of replications)	Growth detection (By streaking)
1-	1/2	1.635 A	1.659 a A	(-ve) growth
2-	1/4	1.550 B	1.570 b B	(-ve) growth
3-	1/8	1.428 C	1.448 c C	(-ve) growth
4-	1/16	1.140 D	1.731 d E	(+ve) growth
5-	Control	1.759 F	2.000 e G	(+ve) growth

Different small letters in a column revealed significant differences (P<0.05) between dilutions factor of bacteriocin. Horizontal different capital letters revealed significant differences (P<0.05) between dilutions of bacteriocin. O.D: Optical density - Ve : No growth + Ve= Growth

References

1. Heechen, W. H. (1996). Bacteriological quality of raw milk: Legal requirement and payment systems . In *Bacteriological quality of raw milk* (pp.1-18). Special Issue No. 9601. Brussels, Belgium:International Dairy Federation.
2. Caprioli, A. S.; Morabito, H.; Brugère, and Oswald,E.(2005).Enterohemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Vet. Res. 36: 289–311.
3. Jo, M.Y. ; Kim, J.H. ; Lim, J.H. ; Kang, MY. ; Koh, H.B. ; Park , Y.H. and et al.(2004). Prevalence of characteristics of *Escherichia coli* O157 from major food animals in Korea. Int. J. Food Microbiol., 95: 41-49.
4. Diels, A. M. J. and Michiels, C. W. (2006). High-pressure homogenization as a non-thermal technique for the inactivation of microorganisms. Critical Reviews in Microbiology. 32: 201-216.
5. 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.

6. Ray, B.(1989).Injured index and pathogenic bacteria . CRC Press, Boca Raton, FL.
7. Gálvez,A.;Abriouel, H.; Benomar, N., and Lucas, R. (2010).Microbial antagonists to food-borne pathogens and biocontrol.Current Opinion in Biotechnology.21:142-148.
8. Masschalck B.; Garcia-Graells C.; Haver E.V. and Michiels C.W.(2000).Inactivation of high pressure resistant *Escherichia coli* by lysozyme and nisin under high pressure.Innovative Food Science and Emerging Technologies .1:39-47.
9. Khudhier,Z.S.(2011).Antibacterial activity of *Lactobacillus acidophilus* bacteriocin against *E.coli* O157:H7 in raw milk.Ph.D.Thesis. Veterinary Medicine Collage, University of Baghdad.
10. Hernnadez, D.; Cardel , E. and Zarate ,V. (2005) . Antimicrobial activity of Lactic acid bacteria isolated from Tenerife cheese initial characterization of Plantaricin TF711 abacteriocin- like substance produced by lactobacillus Plantarum TF711.J.Applied Microbiol .99:77-84 Holt , J.G; Krieg , N.R.
11. Davidson, P. M. and Parish, M. E.(1989). Methods for testing the efficacy of food antimicrobials. Food. Technol., 43: 148-155.
12. Choi , O.K .; Kim , Y.S .; Cho, G.S .and Sung , C.K .(2002). Screening for antimicrobial activity from Korean plants . Kor. J. Food ., 15: 300-306.
13. Feng , P. and Weagant , S.D . (2011) . Diarrheagenic *Escherichia coli* FDA/US Food and Drug administration . Bacteriological Analytical Manual (BAM).
14. Bettelheim, K.A. (1998). Reliability of CHROMagar O157 for the detection of enterohaemorrhagic *Escherichia coli* (EHEC) O157 but not EHEC belonging to other serogroups. J. Appl. Microbiol. 85:425-428.
15. Vernozy-Rozand, C.; Mazuy-Cruchaudet, C; Bavai, C; Montet, MP; Bonin, V; Dernburg, A and Richard, Y. (2005). Growth and survival of *Escherichia coli* O157:H7 during the manufacture and ripening of raw goat milk lactic cheeses. Int. J. Food Microbiol., 105: 83-88 .
16. Lanciotti, R.; Gardini,F, ; Sinigaglia,M. and Guerzoni,M.E.(1996). Effects of growth conditions on the resistance of some pathogenic and spoilage species to high pressure homogenization. Lett. Appl. Microbiol., 22:165–168.
17. MacDonald, F. and Sutherland, A. D. (1993). Effect of heat treatment on *Listeria monocytogenes* and gram-negative bacteria in sheep, cow and goat milks.J.Appl.Bacteriol.,75:336-343.
18. Vachon, J.F.; Kheadr, E.E.; Giasson, J.; Paquin, P., and Fliss,I. (2002). Inactivation of foodborne pathogens in milk using dynamic high pressure J. Food Prot.,65:345-352.
19. Kalchayanand, N.; Sikes, A.; Dunne, C.P. and Ray, B. (1994). Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. Appl. Environ. Microbiol. 60: 4174 – 4177.
20. García-Graells, C.; Masschalck,B. and Michiels,C.W. (1999). Inactivation of *Escherichia coli* in milk by high-hydrostatic pressure treatment in combination with antimicrobial peptides. J. Food Prot. 62:1248-1254.
21. Masschalck, B.; Van Houdt, R.; Van Haver , E.G.R. and Michiels, C.W.(2001). Inactivation of gram-negative bacteria by lysozyme, denatural lysozyme and lysozyme-derived peptides under high hydrostatic pressure . Appl.Environ. Microbiol., 67 (1): 339-344.
22. Kalchayanand, N.; Sikes, A.; Dunne, C.P. and Ray, B. (1994). Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. Appl. Environ. Microbiol. 60: 4174 – 4177.
23. Papagianni, M.; Avramidis, N.; Filioussis, G.; Dasiou, D. and Ambrosiadis, I. (2006).Determination of bacteriocin activity with bioassays carried out on solid and liquid substrates .Microbial Ceel Factories. 5:30.
24. Cos,P.A.J.; Vlietinck, D.V.; Berghe and Maes,L.(2006).Anti-infective potential of natural products., 19:290-302.

التأثيرات التآزرية القاتلة للبكتريوسين مع ضغط التجنيس ضد *الايشريشيا القولونية* O157:H7 في الحليب الخام

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فرع الصحة العامة - كلية الطب البيطري - جامعة بغداد – العراق

الخلاصة

عزلت مستعمرات الأيشيريشيا القولونيةO157:H7 من35 عينة حليب خام و شخصت أعتماداً على تفاعلاتها الكيموحيوية وخصائصها الزرعية و المصلية. أجريت العديد من الفحوصات المصلية على العزلات الأفتراضية لبكتريا الأيشريشيا القولونيةO157:H7 التي تم الحصول عليها من الاوساط الزرعية الأنتقائية التي تشمل وسطي السربيتول ماكونكي (CT-SMAC) و الوسط الصباغي (Chromogenic agar) بحثاً عن المستضد الجسمي و المستضد السوطي بأستعمال أختبار اللا تكس السريع حيث استعملت العده التجارية المتوافرة بهذا النمط المصلي. أن الممارسات غير الصحية المتبعة في أنتاج الحليب في كل من منطقة الذهب الأبيض, أبي غريب, الزيدان، و خان ضاري عكست أنتشارعالي للتلوث بالأيشريشيا القولونية و بمستوى معنوي($P<0.01$) والتي سجلت بنسب80% ,80% ,60%, 60% على التوالي. اثر ضغط التجنيس 1000 باوند/انج^2 و2000 باوند/انج^2 ولخمس دورات معنوياً بمستوى ($P<0.05$) على درجة تثبيط الأيشيريشيا القولونيةO157:H7 في كل من الحليب الكامل و المرق المغذي. تجنيس الحليب بأستخدام ضغط 3000 باوند/انج^2 ثلاث دورات و4000 باوند/انج^2 لدورتين نتج عنه زيادة أضافية للفعالية التثبطية وانخفاض معنوي اضافي على مستوى($P<0.05$) للأيشيريشيا القولونية O157:H7. تحقق القضاء الكامل على الأيشيريشيا القولونية O157:H7 عند تجنيس الحليب الكامل بمستوى ضغط 5000 باوند/ أنج^2 لدورة واحدة. أستعمل الأختبار الحيائي للأنتشار في الحفر عبر الأكار لتقييم الفعالية التثبطية للبكتروسين الخام المنتج من السلالة القياسية *L. acidophilus* LA-K ضد الأشيريشيا القولونية O157:H7 . جرثومة الأيشيريشيا القولونية المعوية النزفية O157:H7 أبدت مقاومتها للبكتيروسين الخام أذا أنها لم تظهر أي منطقة تثبيط حول اي حفرة عوملت بالبكتريوسين. كان معدل قطر منطقة تثبيط البكتروسين الخام للأيشيريشيا القولونية O157:H7 المجهدة بأستعمال الضغوط 4000 باوند/انج^2, 3000 باوند/انج^2, 2000 باوند/انج^2 , 1000 باوند/انج^2 (14مليمتر , 12مليمتر , 10 مليمتر و 8 مليمتر)على التوالي. المستوى المتوسط أو العالي لضغط التجنيس أثر معنوياً و بمستوى ($P<0.05$) على درجة تثبيط البكتروسين الخام للأيشريشيا القولونية O157:H7 المجهدة بأستخدام الضغط. تم أيجاد الفعالية التثبطية للبكتروسين الخام بأستخدام الطريقة الضوئية أو طريقة قياس العكرة. أشارت النتائج الى عدم ظهور أي نمو مع عدم وجود أي عكرة مرئية لبكتريا الأيشريشيا القولونية O157:H7 في داخل المرق المغذي المدعم بالبكتروسين المخفف1/2 , 1/4 , 1/8. نتج عن تخفيف البكتريوسين الى 1/8 الى عدم ظهور اي عكرة مرئية بعد الحضن حتى الصباح عند درجة حرارة 37 م° والذي أعطى قراءة للكثافة الضوئية 1.448 .

الكلمات المفتاحية: التأثيرات التآزرية, البكتريوسين ,التجنيس, الايشريشيا القولونية O157:H7, الحليب الخام.