ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
November 2015 Vol.:4, Issue:4

Characterization of Purified Bacteriocin (Plantaricin and Acidocin) Produced from *Lactobacillus* Isolates and Study its Effects Against Growth Pathogenic Bacteria

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

Essam F.Al-Jumaily[1], Rana H. Raheema[2] and Hassan A. Abdul-Ratha[3]

[1]Institute for Genetic Engineering and biotechnology for Graduate Studies – Baghdad University
[2]Microbiology Department-college of Veterinary Medicine –Wasit University
[3]Microbiology Department-college of Veterinary Medicine –Baghdad University, Iraq.

Submission: 11 November 2015
Accepted: 15 November 2015
Published: 25 November 2015

Keywords: Molecular weight, plantaricin, acidocin, thermostability, cell permeability

ABSTRACT

The plantaricin and acidocin production was induced by adding the mutagenic agent Mitomycin C. The physical characteristics were also studied and the results showed that the molecular weight of plantaricin and acidocin were 14.13, 31.62 KD .respectively by gel filtration chromatography. Plantaricin activity was stable at pH values (3-9) but 50% of its activity was lost at pH 10, while in acidocin activity was stable at pH values (3-7) but 50% of its activity was lost at pH 9. Plantaricin showed high thermostability at different temperatures (50-75)°C for (10-60) min , it remained active after being treated with 100°C for (10-30) min, but it retained only 50% of its activity after exposure to 100°C for (60) min and autoclaving at (121°C for 15 min), while acidocin showed high thermostability at different temperatures (50-100)°C for (10-60) min , but it retained only 50% of its activity after treatment at autoclaving at (121°C for 15 min).The antibacterial activity was observed that crude plantaricin and acidocin has a bactericidal effect against *Escherichia coli* , *Salmonella typhimurium*, *Listeria monocytogenes* and *Staphylococcus aureus* when the numbers of cells were decreased with increasing of plantaricin and acidocin concentration to 320 AU/ml. The lytic activity of pure plantaricin and acidocin was studied, and the results showed that pure plantaricin and acidocin possess the lytic activity on cell membrane, when the absorbance at 600nm decreased for *Escherichia coli* , *Salmonella typhimurium*, *Listeria monocytogenes* and *Staphylococcus aureus* with increasing of plantaricin and acidocin concentration to 320 AU/ml. The effect of plantaricin and acidocin on the cell permeability was studied and the results showed that the absorbance at 260nm increased, indicating that DNA leaked from cells due to the cell lysis.

INTRODUCTION

Bacteriocins are proteinaceous compound which have inhibitory effects towards sensitive strains produced by both gram-positive and gram-negative bacteria, Bacteriocins producing lactic acid bacteria are used in food fermentations especially in dairy products. In USA, only nisin produced by *Lactobacillus lactis* has been permitted as a food preservative, It has also been used in health care products and cosmetics for treatment of acne, They are also being used in toothpaste and mouthwash for the inhibition of dental caries and periodontal diseases[1]. Bacteriocins are polypeptides , with bactericidal or bacteriostatic activity, against those bacteria which are closely related to the producer strain. The bacteriocins produced by gram positive bacteria, in particular, the lactic acid bacteria display fairly broad inhibitory spectra with food preservative and therapeutic potentials[2]. The aim of this study role of bacteriocin produced by lactic acid bacteria on the pathogenesis of Enteropathogenic *E. coli* (EPEC) (*in vivo*) and the synergistic effect of plantaricin and acidocin against cell permeability.

MATERIALS AND METHODS

Induction for Plantaricin and Acidocin production

The qualified producing strains (*L. plantarum and L. acidophilus*) was inoculated into the BGM with Mitomycin C (2 μg per ml) then incubated at optimal temperature for optimal time. The antimicrobial activity of plantaricin and protein concentration was determined according to Pilasombut[3]. Pure plantaricin and acidocin were obtained according to Al-Juamily[4].

Characterization of plantaricin and acidocin

In vitro antibacterial activity of Crude Plantaricin and Acidocin

Plantaricin and Acidocin were examined for inhibitory activity against different strains of bacteria using the Agar Well Diffusion (AWD) assay [5].

Estimation of molecular weight

Molecular weight of Plantaricin and Acidocin was determined by gel filtration chromatography using Sepharose 6B column [6].

Effect of pH on plantaricin, Acidocin stability (Ali, 2010)

The effect of pH on Plantaricin, Acidocin activity was analyzed by using HCl and NaOH for maintaining the pH of 3,4,5,6,7,8,9,10 and 11, After 30 minutes of incubation at 30°C, 37°C and tested for remaining activity. It was calculated as follows:

$$\text{Residual activity \%} = \frac{\text{Remaining units}}{\text{Original units}} \times 100$$

Effect of Temperature on plantaricin, Acidocin stability

The effect of temperature on plantaricin, Acidocin activity was assayed by heating the plantaricin solution to (50, 75, and 100) °C respectively. Plantaricin, Acidocin activity was assayed after (10, 30 and 60) minutes at each of these temperatures. Activity also assayed after 15 minutes at 121°C.

Antibacterial Activity of Crude (Plantaricin and Acidocin)

Effect of Crude (Plantaricin and Acidocin) on Cell Lysis and Viability[7].

The effect of crude plantaricin & Acidocin on indicator bacteria *E. coli, S. typhmurium, L . monocytogens* and *S. aureus*. The inhibition rate was calculated as:

$$\text{Inhibition rate (\%)} = \frac{\text{No. of cells in zero time} - \text{No. of cells in required time}}{\text{No. of cells in zero time}} \times 100$$

Synergistic effect of pure (Plantaricin and Acidocin) on Cell Permeability of *E.coli, S. typhmurium, L. monocytogens* and *S.aureus*[8].

RESULTS AND DISCUSSIONS

Effect of Mitomycin – C on Inducing Plantaricin and Acidocin

The results effect of Mitomycin – C on inducing Plantaricin and Acidocin showed increased protein concentration and specific activity with used of Mitomycin – C from *Lactobacillus plantarum* and *Lactobacillus acidophilus.*

The results came in agreement with Laftah,[8] who used Mitomycin –C with 2 µg/ml for induced Klebocin from *Klebsiella pneumonia.*

Characterization of Plantaricin and Acidocin

***In vitro* antibacterial activity of Plantaricin and Acidocin**

The results in table (1) and Figures (1, 6) showed that Plantaricin and Acidocin of the *Lactobacillus plantarum* and *Lactobacillus acidophilus* have an antibacterial activity against the growth of different gram positive and gram negative bacteria, the highest inhibition zone diameter of Plantaricin (49.33 ± 0.29) for *Klebsiella* while (49.66± 0.29) with *Citrobacter* Spp, the lowers inhibition zone diameter was (33 ± 0.86) for *Bacillus* Spp ,while in case of Acidocin the highest inhibition zone diameter (49.33 ± 0.29) for *Proteus* Spp and *Streptococcus* Spp, the synergistic effect between Plantaricin and Acidocin recorded highly antibacterial activity with highest inhibition zone diameter (49.66 ± 0.29) for *Proteus* Spp and lowers inhibition zone diameter for *Staph aureus* .

The antibacterial activity of Plantaricin against EPEC showed (40 ± 0.0) while, in case of acidocin recorded (45 ± 0.0), synergistic effect between Plantaricin and Acidocin gave (45 ± 0.0). The statistical analysis showed that there was a significance (P≤0.05) difference between the crude Plantaricin and crude Acidocin with (45 ± 0.0) against the growth of EPEC isolates.

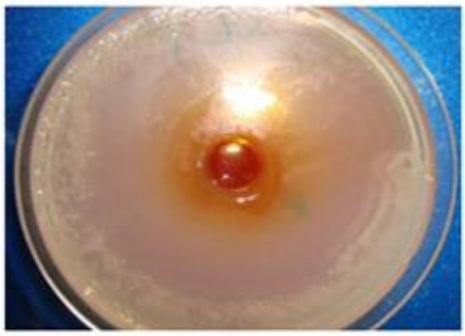

Figure (1): Antibacterial effect of crude plantaricin against *Clostridium* spp

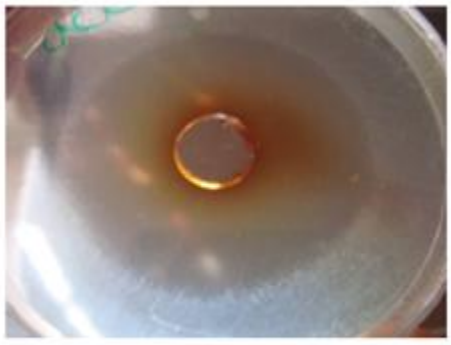

Figure (2) : Antibacterial effect of crude acidocin against

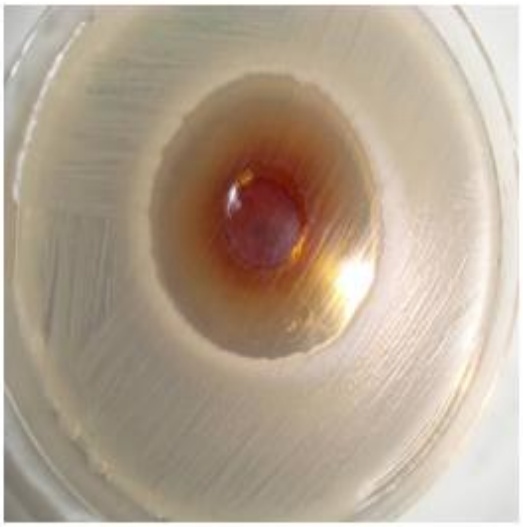

Figure (3): Antibacterial effect of crude acidocin against *Proteus* spp

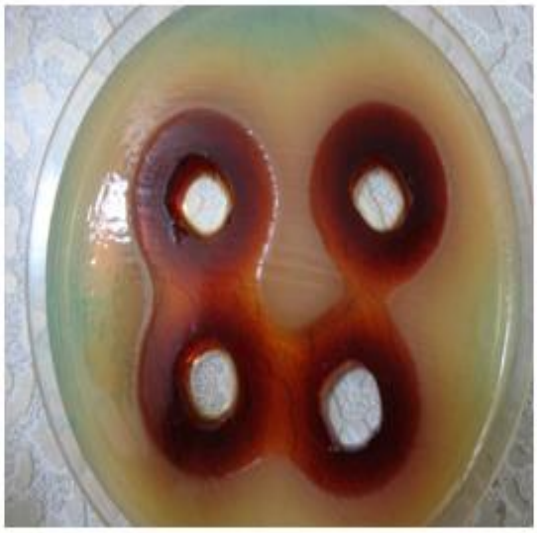

Figure (4): Synergistic effect of crude plantaricin and acidocin against *Listeria monocytogens*)

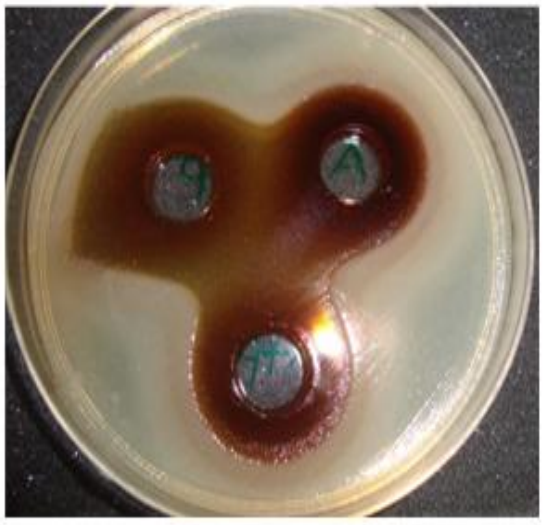

Figure (5) : Antibacterial effect and synergistic effect of Plantaricin and Acidocin against *Salmonella typhimurum*

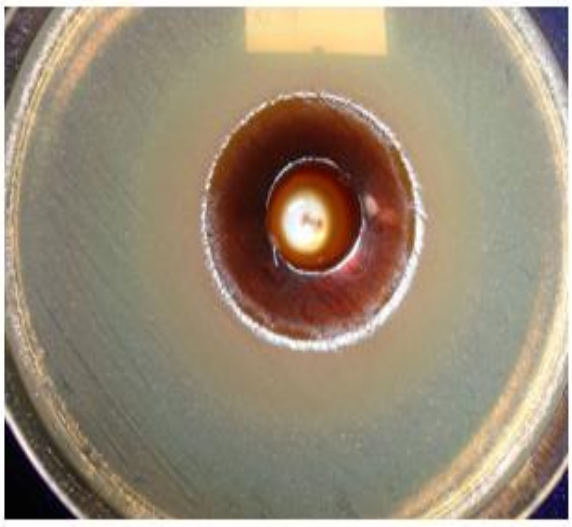

Figure (6) : Antibacterial effect of crude acidocin against *Staph aureus*, with diameter of well (6 mm)

Table (2): Antibacterial effect of Partial purified (Plantaricin and Acidocin)

Bacteria	Plantaricin	Acidocin	Plantaricin & Acidocin	DMSO
E. coli	b 40 ± 0.0 B	b 45 ± 0.0 A	b 45 ± 0.0 A	0±0 C
Proteus Spp	b 40 ± 1 B	a 49.33 ± 0.29 A	a 49.66 ± 0.29 A	0±0 C
Bacillus Spp	c 33 ± 0.86 C	c 42 ± 0.0 B	b 45.33± 0.29 A	0±0 D
Pseudomonas	b 42. 33 ± 1.26 C	a 48. 66 ± 0.58 A	b 45 ± 0.0 B	0±0 D
Clostridium	a 48.66 ± 0.58 A	bc 44.33 ± 0.29 B	a 48.66 ± 0.58 A	0±0 C
L. monocytogens	b 43.66 ± 0.58 A	d 35 ± 0.0 B	b 45 ± 0.5 A	0±0 C
Staph. aureus	c 34.66 ± 0.29 B	d 34.66 ± 0.29 B	c 37.33 ± 0.29 A	0±0 C
Neisseria meningitides	a 47.33 ± 0. 29 A	b 45 ± 0. 0 B	a 49 ± 0.5 A	0±0 C
Streptococcus	b 41.33 ± 0.58 C	a 49.33 ± 0.29 A	b 45.66 ± 0.58 B	0±0 D
Klebsiella	a 49.33 ± 0.29 A	d 30 ± 0.0 B	ab 47.66 ± 0.58 A	0±0 C
Citrobacter Spp	a 49.66 ± 0.29 A	b 45 ± 0.5 B	a 49 ± 0.0 A	0±0 C
Salmonella typhimurum	b 40.66 ± 0.58 B	c 42 ± 0.86 B	b 45.66 ± 0.29 A	0±0 C

Different small letters revealed significant differences between the group of bacteria for the same bacteriocin at significant level ($p \leq 0.05$).

Different capital letters revealed significant differences between the group of bacteriocin for the same bacteria at significant level ($p \leq 0.05$).

Estimation of molecular weight

Figure (7) showed that the molecular weight is determined by gel filtration in Sepharose - 6B based on the standard curve made by standard proteins for activity against the indicator strain *Escherichia coli* was used for estimation of molecular weight of plantaricin and acidocin by gel filtration chromatography with the aid of fraction of standard proteins showed that the resolute protein had (14.13 and 31.62) KD molecular weight respectively.

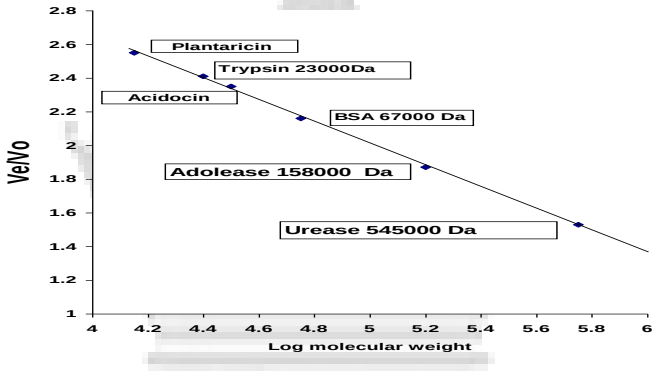

Figure (7): Determination of the molecular weight of purified Plantaricin and Acidocin of the local isolate *Lactobacillus plantarum* & *Lactobacillus acidophilus* by gel filtration chromatography using standard proteins of different molecular weights.

pH Stability for Plantaricin and Acidocin

Results in figure (8) shown that purified plantaricin was stable at pH values 3,5,7 and 9 for purified plantaricin while purified acidocin was stable at pH values 3,5,7. At these values plantaricin remained active while at pH values 10, the plantaricin lost 50% of its activity. Whole activity of plantaricin was lost at the pH values 11, indicating its sensitivity to alkali treatment. These results agreed with the Ali[7] who showed that Plantaricin VGW8 was recorded high stability at wide range of pH values (3-9). In case of purified acidocin at pH value 9, the acidocin lost 50% of its activity. Whole activity of acidocin was lost at the pH values 10 and 11 , this

results agreed with the Moghaddam,[11] who showed that bacteriocin of *Lactobacillus acidophilus* is stable at pH range of 3-10.

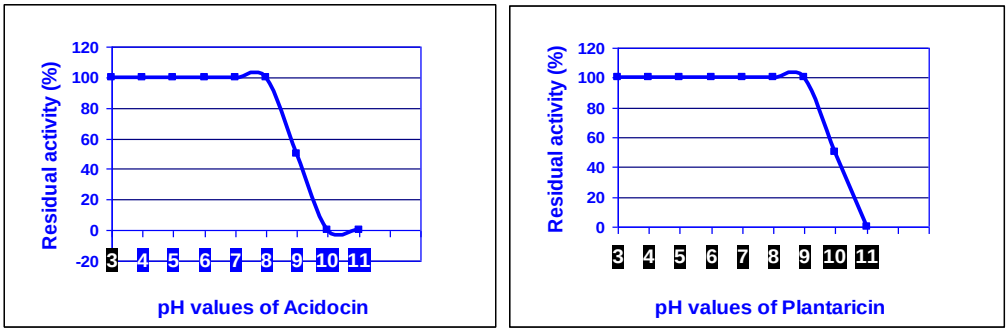

Figure (8): Stability of Plantaricin and Acidocin produced by *Lactobacillus plantarum* and *Lactobacillus acidophilus* at different pH values.

Thermostability for Plantaricin and Acidocin

Thermostability of purified plantaricin and acidocin was assayed at different temperatures. As shown in figure (9), the plantaricin was resistant to treatments with (50,75)°C for (10,30 and 60) min, respectively. At 100°C for (10 and 30) min, respectively, plantaricin was also appeared thermostability. However, 50% activity was lost after 60min at 100°C and after autoclaving (121°C /15min) this result is in agreement with (13) who reavel that Plantaricin produced by *L. plantarum* is a thermostable, while in case of acidocin was resistant to treatments of (50,75)°C for (10,30 and 60) min, respectively. At 100°C for (10, 30 and 60) min, respectively, acidocin was also appeared thermostability. However, 50% activity was lost after autoclaving (121°C /15min).

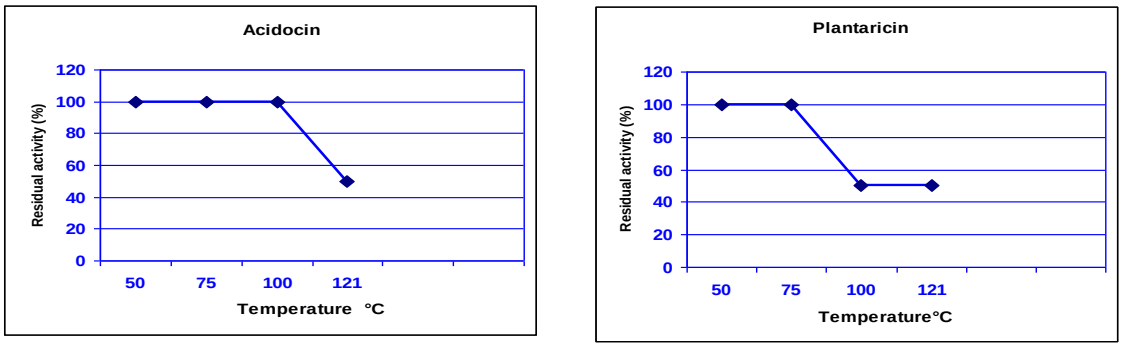

Figure (9): Residual activity of purified Plantaricin and Acidocin at different temperatures for 60 minutes except 121°C for 15 minutes.

Antibacterial Activity of Plantaricin and Acidocin

Effect of Plantaricin and Acidocin on Viability and Lysis of Indicator Bacteria

The addition of 320 AU/ml of plantaricin and acidocin to *E. coli*, *S. typhmurium*, *L . monocytogens* and *S.aureus*resulted in a marked antibacterial effect with a reduction of cells count of the treated sample after one hour, two hours, 4 hours and 24 hours with different inhibition rate as shown in table (2).

At 4 hours of incubation with 320 AU/ml, the complete killing of *E.coli* , *S. typhmurium* , *L . monocytogens* and *S. aureus* cells were observed at 320 AU/ml concentrations for plantaricin, while in case of acidocin the complete killing of *E. coli* , *S. typhmurium* , *L. Monocytogens* happened at this time, except *S. aureus* which recorded complete killing after 4 hours. In cases of 160 AU/ml, 80 AU/ml for plantaricin and acidocin the complete killing after 4 hours and at 24 hours in both concentration, the complete killing, indicating bactericidal mode of action of plantaricin and acidocin.

Table (2): Inhibition rate of effect of Plantaricin and Acidocin with different concentration on indicator bacteria at different periods A (Plantaricin), B (Acidocin) (A)

Concentration / Indicator Bacteria	320 AU / ml			160 AU / ml			80 AU / ml		
	1 hour	2 hours	4 hours	1 hour	2 hours	4 hours	1 hour	2 hours	4 hours
E. coli	99.90%	99.99%	100 %	99.04%	99.10%	99.92%	92.65%	99.54%	99.95 %
S. typhmurium	99.98 %	99.99%	100 %	98.75%	99.88%	99.98%	80.66%	99.80%	99.93 %
L. monocytogenes	99.91%	99.99%	100 %	98..86%	99.90%	99.98%	92.71%	99.47 %	99.91 %
S. aureus	99.90%	99.99%	100 %	99.54%	99.92%	99.99%	88.31%	99.51%	99.94 %

(B)

Concentration Indicator Bacteria	320 AU / ml			160 AU / ml			80 AU / ml		
	1 hour	2 hours	4 hours	1 hour	2 hours	4 hours	1 hour	2 hours	4 hours
E. coli	99.54%	99.98%	100 %	98.04%	99.91%	99.99%	88.22%	99.21%	99.91 %
S. typhmurium	99.80 %	99.99%	100 %	98.11%	99.90%	99.99%	81.33%	99.21%	99.88 %
L. monocytogenes	99.90%	99.98%	100 %	98.71%	99.92%	99.99%	89.66%	99 %	99.98 %
S. aureus	99.51%	99.94%	99.99%	90.51%	99.04%	99.94%	83.63%	97.19%	98.25 %

Effect of Plantaricin and Acidocin on Cell Permeability

Spectrophotometric method was used to investigate the effect of plantaricin and Acidocin on cell permeability. As shown in table (3), absorbance at 260nm was 0.530, 0.472, 0.432 and 0.511 for untreated cells of *E. coli*, *S. typhmurium*, *L. monocytogens* and *S. aureus* respectively. Whereas the treated cells of the same indicator bacteria increased in absorbance to 3, 1.98, 2.34 and 2.88 for treated indicator cells indicating that DNA leaked from these cells of indicator bacteria due to the cell lysis.

Table 3: Extracellular levels of DNA recorded after treatment of Indicator bacteria with Plantaricin and Acidocin

Treatment	Absorbance at 260nm			
	A	B	C	D
Treated cells	3	1.98	2.34	2.88
Untreated cells	0.530	0.472	0.432	0.511
Plantaricin and acidocin, no cells	0.557	0.557	0.557	0.557

A= *E. coli* ***B***= *S. typhmurium* ***C*** = *L . monocytogenes* ***D***= *S. aureus*

REFERENCES

1. Indira , K.; Jayalakshmi , S.; Gopalakrishnan .A . and Srinivasan , M.Biopreservative potential of marine *Lactobacillus* Spp. African journal of Microbiology Research Vol. 5 (16), pp.2287 – 2296.2011.
2. Lipton , A.P .; Sarika,A.R. and Aishwarya, M.S. Bacteriocin production by a new isolate of *Lactobacillus rhamnosus* GP1 under different culture conditions .Advance Journal of Food Science and Technology 2 (5): 291-297.2010.
3. Pilasombut,K.;Sakpuaram,T.;Wajjwalku,W.;Nitisinprasert,S.;Swetwiwathana,A.;
Zendo,T.;Fujita,K.;Nakayama,J.andSonomoto,K.Purification and amino acid sequence of a bacteriocins produced by *Lactobacillus salivarius* K7 isolated from chicken intestine.Sonklanakarin.J.Sci.Technol.28(1):121-131.2005.
4. Al-Jumaily, E.F. ; Hassan A. Abdul-Ratha, H.A. and Raheema,R.H. Purification of Acidocin from *Lactobacillus* isolates on pathogenesis of Enteropathogenic*E.coli.* Online International Interdisciplinary Research J. Vol.II, Iss.V. pp. 2-8.2012.
5.Lasta,S .Z.;Fajioun ,H.;Darbon ,p.;Mansuelle ,N.;Andreotti ,J.;Sabatie,L.;Abdeilatif ,A.;Boudabous and Sampieri,F.Chemical synthesis and characterization of J46 peptide , an atypical class IIabacteriocin from Lactococcuslactissubsp.cremoris J46 strain . J.Antibiotics , 61:89-93. 2008.
6.Sanni, A.I.; ogunbanwo, S.T. and Onilude, A.A. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillusbrevis* OG1. Afr. J. Biotechnol., 2(8): 219 – 227.2003.
7.Ali , W.S. Production, Purification and Characterization of Plantaricin from Local Strains of *Lactobacillus plantarum* . Ph.D.thesisCollege of Science, University of Baghdad .2010.
8. Kandela, N.J.N. Production, purification and characterization of enterocinfrom *Enterococcusfeacalis* local isolates from different clinical sources. Ph.D thesis, Al-Mustansiriya University, College of Science.2006.
9. Todorov, S.D. Bacteriocins from *Lactobacillusplantarum* – production, genetic organization and mode of action. Braz. J. Microbiol., 40 : 209 – 221.2009.
10. Laftah, A.R.Effect of klebocin partially purified from bacteria *Klebsiella pneumoniae* Local Isolated on some enteric bacteria & on some immune cells.Athesis submitted to the college of science, Al-Mustansiriyah University in partial fulfillment of the Requirements for the Degree of Master of Science in Biology/ Microbiology. 2010.
11. Moghaddam,M.Z.;Satari,M.;Mobarez,A.M.;Doctorzaden,F. Inhibitory effect of yogurt Lactobacilli bacteriocins on growth and verotoxins production of enterohemorrhagic*Escherichia coli* O157:H7 .Pak J . Biol .Sci . 9(11),2112-2116.2006.