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Antibacterial activity of crud Bacteriocin- like substance against food borne bacterial pathogens

Alyaa Razooqi Hussein*, Zainab Zamel Khalaf, Zainab Samir, Russul Samir

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

Abstract

Lactobacillus is one of Lactic Acid Bacteria group, they are known to exhibit antagonistic activity against pathogenic organisms. This study evaluates the protective effect of *Lactobacillus acidophilus* Crud Bacteriocin like substance against growth of some food borne pathogenic bacteria. Antimicrobial effect of crud bacteriocin like substance was tested against the growth of *S. typhi* and *S. aureus* that isolated from food samples by agar well diffusion assay.

The results showed that the crud extract of bacteriocin like substance had antibacterial activity against the two tested bacteria, and the effect against *S. typhi* was greater than that of *S. aureus*, the zone of inhibition was (15)mm against *S. typhi* and (12)mm against *S. aureus*. The antibiotic susceptibility test was also studied against the two foodborne pathogenic bacteria. This study aims to screen the antibacterial activity of crud bacteriocin like substance from lactic acid bacteria against two food borne pathogens.

Keywords: *Lactobacillus acidophilus*, Bacteriocin like substance, foodborne pathogenic bacteria

الفعالية ضد بكتيرية لمادة خام شبيهه بالبكتريوسين ضد ممرضات بكتيرية منقولة بالغذاء

علياء رزوقي حسين*، زينب زامل خلف، زينب سمير، رسل سمير

قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.

الخلاصة

اللاكتوباسلاس هي واحدة من مجموعة بكتريا حامض اللاكتيك، و هي معروفة بأظهار الفعالية المضادة لبعض الكائنات الممرضة. قيمت هذه الدراسة التأثير الوقائي لمادة خام تشبه البكتريوسين من بكتريا لاكتوباسلاس اسيدوفلس ضد نمو بعض البكتريا الممرضة المنقولة بالاغذية. اختبر تأثير المادة الخام الشبيهة بالبكتريوسين ضد مايكروبي ضد نمو بكتريا السالمونيلا تايفي والعنقوديات الذهبية المعزولة من عينات الاغذية باستخدام فحص الانتشار في حفر الاجار. اطهرت النتائج بان للمستخلص الخام للمادة الشبيهة بالبكتريوسين فعالية ضد بكتيرية ضد كلا النوعين من البكتريا المختبرة وكان التأثير ضد السالمونيلا تايفي اكثر مما كان ضد العنقوديات الذهبية، منطقة التثبيط كانت 15 ملم ضد السالمونيلا تايفي و 12 ملم ضد العنقوديات الذهبية. وكذلك درس اختبار الحساسية للمضادات الحيوية لكلا النوعين الممرضين المنقولين بالاغذية. تهدف هذه الدراسة لفحص الفعالية ضد بكتيرية لمادة خام شبيهة بالبكتريوسين من بكتريا حامض اللاكتيك ضد ممرضين بكتيريين منقولين بالاغذية.

*Email: alyatiba@yahoo.co.uk

Introduction

Bacteriocins are biologically active peptides produced by several bacterial species especially by *Lactobacillus spp.* and are active against both gram positive and gram negative pathogenic bacteria [1]. Lactic acid bacteria (LAB), against which the producer has a specific immunity mechanism [1]. Bacteriocins are emerging such as use in functional foods and use in human therapy as an alternative to antibiotics. Bacteriocins antimicrobial properties are aimed at stimulating the immune system, It can be used as an aid treatment of gastrointestinal and urinary tract diseases [2]. *Lactobacilli* are characterized by their ability to inhibit the growth of bacteria throughout the production of antimicrobial materials such as bacteriocins and biosurfactants, thus preventing the formation of biofilms [3, 4].

Antimicrobial effects of lactic acid bacteria are formed by producing some substances such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins [5].

In recent years, interest in bacteriocins has grown substantially due to their potential usefulness as natural food preservatives in addition to promoting good health [6]. The widespread use of antibiotics in food animal production systems has resulted in the emergence of antibiotic resistant zoonotic bacteria that can be transmitted to humans through the food chain. Infection with antibiotic resistant bacteria negatively impact on public health, due to an increased incidence of treatment failure and severity of disease. Development of resistant bacteria in food animals can result from chromosomal mutations but is more commonly associated with the horizontal transfer of resistance determinant borne on mobile genetic element [7].

The aim of this study was to determine the antibacterial effect of crud bacteriocin-like substance produced from *L.acidophilus* against food borne bacterial pathogens isolated from local food samples that might be resistant to some antibiotics.

Materials and Methods

Bacterial strains

Lactobacillus acidophilus isolated from yogurt, obtained from Faculty of Veterinary Medicine/University of Baghdad.

Isolation and Identification of pathogenic bacteria from local food samples

Ten samples of hash meat and 10 samples of chicken meat (poultry) were collected in sterile containers until using. Five grams of each food sample suspended in 45ml of sterile peptone water then use rinse and washing method then inoculate 0.1 ml into mannitol salt agar (for *Staphylococcus* isolation) and 0.1ml into Salmonella-Shigella agar (for *Salmonella* isolation) by using pouring plate method then incubate the plates at 37°C for 24 hr. The isolates were identified by morphological and biochemical tests, (gram reaction) (Indol, methylene red test, voges-proskauer test, citrate utilization test (IMViC test) S.S agar (selective & differential), MacConkey agar (lactose non fermenter) L.N.F, TSI, sugar fermentation tests (mannitol, glucose) for *Salmonella*, blood agar (hemolysis), nutrient agar for catalase activity, mannitol salt agar (selective and differential) for *Staphylococcus*.

Bacteriocin-like substance Production from *L.acidophilus*

L.acidophilus was grown in MRS broth at 37°C for 48hr. anaerobically. After incubation, the broth was centrifuged at 5000 rpm for 10 min. and the cells were separated out and adjusted to pH 6.0 using 1N NaOH. The cell free supernatant was used as crud bacteriocin-like substance and kept in refrigeration till use [8].

Antimicrobial activity of bacteriocin-like substance

Antimicrobial activity of bacteriocin-like substance against pathogenic microorganisms was determined by agar well diffusion method under aerobic condition [8]. The two isolates of *Salmonella* & *Staphylococcus* were cultured in Brain-heart infusion broth (incubate at 37°C for 24 hr.) then compare the microbial growth with MacFarland tube [0.5] (1×10^8 cell/ml) in order to get a calculate intensity of growth, the inhibitory activity against pathogenic M.O. was tested on Muller-Hinton agar by culturing these two sample on this media by swabbing, wells (6mm) were cut in Muller-Hinton agar plate by using sterile cork borer and 0.1ml of cell free culture supernatant (crud bacteriocin) was added into each well. Plates were incubated at 37°C for 24 hr. The antimicrobial activity was determined by measuring the diameter of the inhibition zone around the wells by ruler in mm.

Antibiotic susceptibility test of foodborne pathogens

Antimicrobial susceptibility testing for *S.typhi* & *S.aureus* was performed using Kirby-Bauer disk diffusion method on Muller Hinton agar, then the plates incubate at 37°C for 24 hr. The antibiotics that used from Turkish company "Bioanalyse" were in Table-1.

Table 1- The antibiotics that used in this study

<i>S.typhi</i>	<i>S.aureus</i>
Ampicillin 10µg (AM)	Nalidixicacid 10µg (NA)
Chloramphenicol 30µg (C)	Gentamicin 10µg (CN)
Tetracyclin 10µg (TE)	Kanamycin 30µg (K)
Streptomycin 10µg (S)	Cephalothin 30 µg (KF)

Statistical Analysis

SPSS v24 was used in analysis of data statistically, Independent Samples Test used in the analysis of antimicrobial activity of crud bacteriocin against two food borne pathogens, The Least Significance Difference (LSD) was determined by one-way analysis of variance (ANOVA) in the analysis of the effect of antibiotics against the two tested bacteria separately. All experiments were carried out in duplicates.

Results and Discussion

Diagnosis of the isolates

Two pathogenic isolates were obtained *Staphylococcus aureus* from hash meat and *Salmonella typhi* from chicken meat. The results appeared that isolated bacteria belong to *Staphylococcus* which is G+ve cocci, arranged in clusters, non-motile, non spore former, having yellow colonies on mannitol salt agar as in Figure-1 which is selective because it contains salt and differential because it distinguish between mannitol fermenter and mannitol non fermenter, type of hemolysis shows complete β-hemolysis on blood agar as in Figure-2, catalase test is positive, appear like bubble after adding hydrogen peroxide (H₂O₂) Figure-3, all these tests prove that this isolate is *Staphylococcus aureus*.

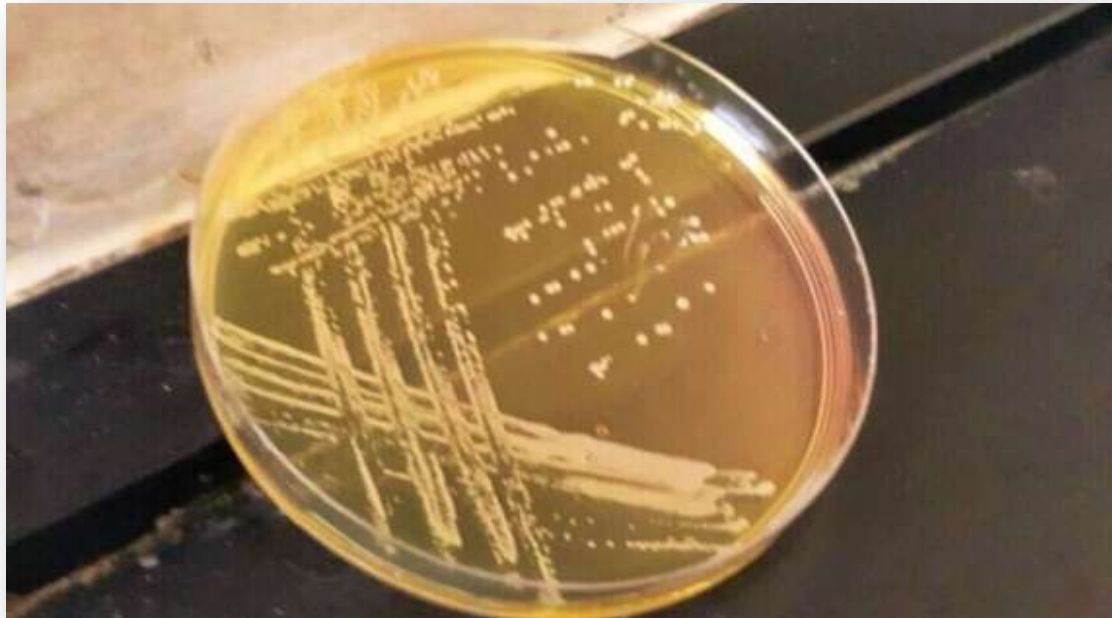


Figure 1- *S.aureus* colonies on mannitol salt agar



Figure 2- complete β - hemolysis of *S.aureus* on blood agar.



Figure 3- positive result of catalase test *S.aureus* (catalase +ve).

Salmonella is G^{-ve} bacilli non-spore former facultative anaerobes, it appears like black colonies on S.S agar as in Figure-4 which is selective because it contains bile salt for G⁺ve inhibition and G^{-ve} other than enterobacteriaceae, differential because it contains Na-thiosulfate & ferric citrate for H₂S production, lactose non fermenter, TSI test K/A ++ as in Figure-5 and motility test (+), IMViC test MR (+), VP(-) as in Figure-6, citrate utilization (-) as in Figure-7, and sugar fermentation tests (mannitol and glucose) +ve for *S.typhi* as in Figure-8.



Figure 4- *S.typhi* colonies on S.S agar.

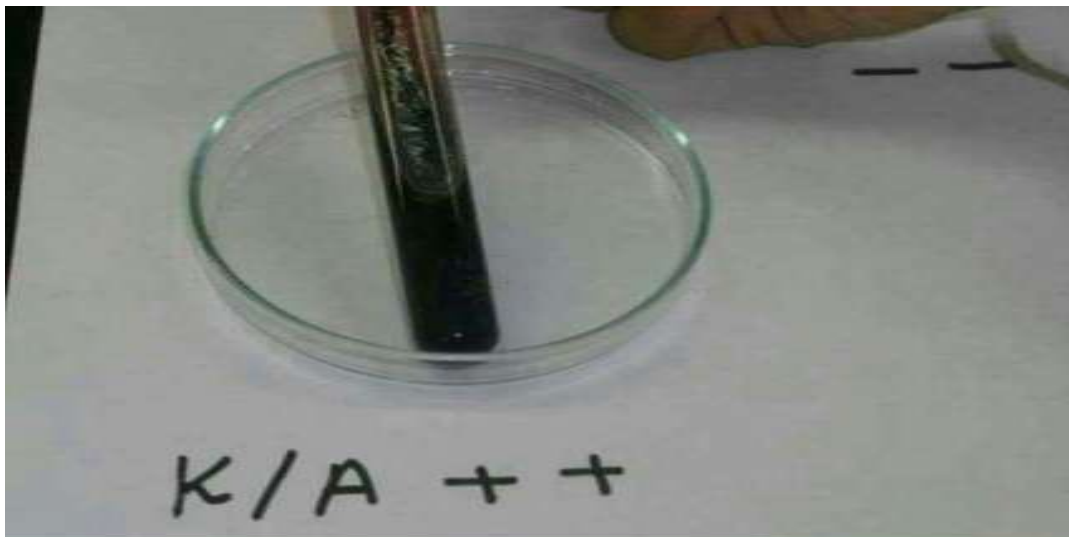


Figure5-TSI test for *S.typhi*

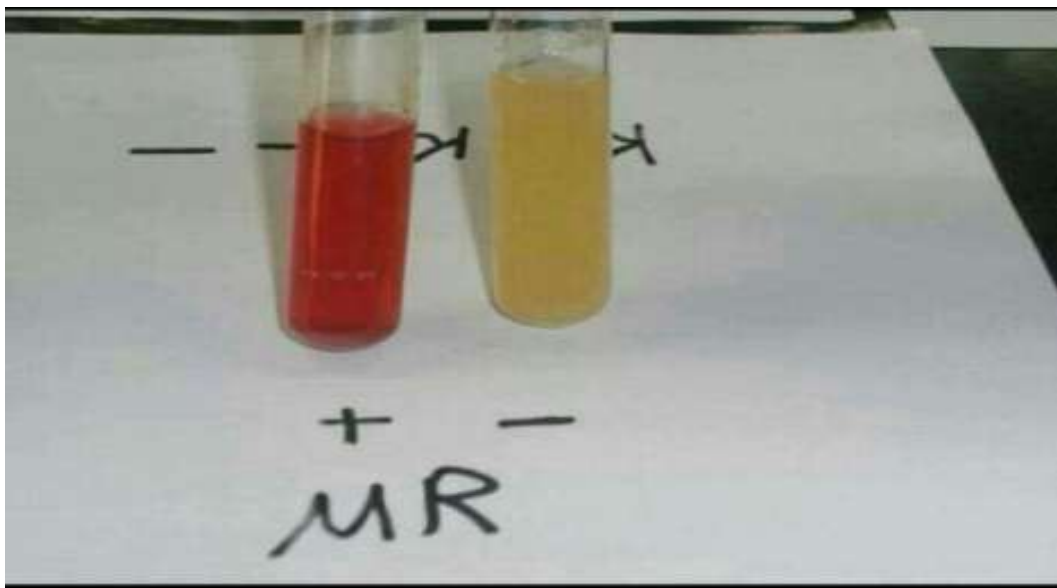


Figure 6-MR, VP tests for *S.typhi*



Figure 7- result citrate utilization test

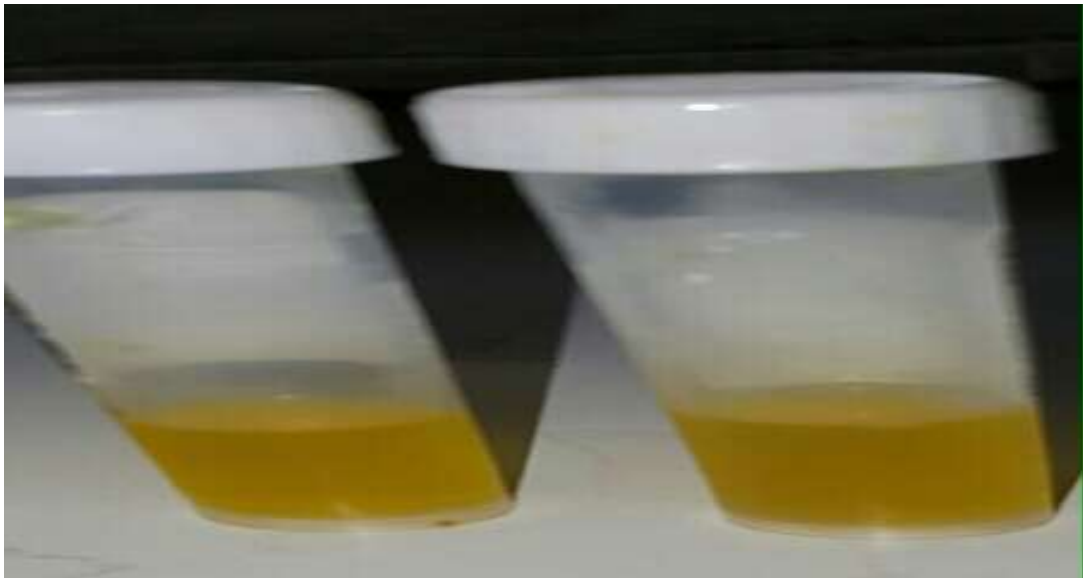


Figure 8- + result for sugar fermentation tests of *S.typhi* .

Antimicrobial activity of crud bacteriocin-like substance

An agar well diffusion method was used to assess the production of antimicrobial compounds by the selected isolate from yogurt against 2 pathogens. The result showed that the crud extract of bacteriocin from *L.acidophilus* has antibacterial activity against the two tested bacteria. The inhibition zone was (15) mm against *S.typhi* and (12) mm against *S.aureus* as shown Table-2

Table 2- Antimicrobial activity of crud bacteriocin against two food borne pathogens

strain	Inhibition zone of crud bacteriocin-like substance
<i>S. aureus</i>	12±1.4 mm
<i>S. typhi</i>	15±1.4 mm

From the result above it show that crud bacteriocin-like substance has inhibitory effect on *S.typhi* more than that of *S.aureus*, Our result were in agreement with Debapriya and Pratima [9] ,bacteriocin producing *Lactobacillus spp.* Isolated from yoghurt pH(5), showed wide range of antimicrobial

activity against some major food borne pathogen. Local research [10] showed that *Proteus mirabilis* bacteriocin had higher effect against *Klebsiella* than the effects of it against other bacterial species in premature and mature biofilm.

Antibiotic susceptibility test of food borne pathogens

The result showed that *S.aureus* was sensitive to Nalidixic acid and Kanamycin, but this bacteria was resistant to Cephalothin and moderate in resistant to Gentamycin as shown in Tables-(3,4 and 5) all antibiotics used had asignificant effect ($P<0.05$) against *S.aureus*. While *S.typhi* was sensitive to Chloramphenicol and tetracycline, but it is moderate in resistant to Streptomycin, also this bacteria was resistant to ampicillin as shown in Tables(6,7 and 8)also all antibiotics used had asignificant effect ($P<0.05$) against *S.typhi* ,this result was inagreement with [11] since the researcher indicates that in Romania their was an increasing emergence of antibiotic resistance among pathogenic bacterial strain in poultry, the significant usage of Tetracycline and Ampicillin in poultry production, it is therefore important to examin Tetracyclin and Ampicillin resistance marker since they are associated with transposable multidrug resistant elements [12].

Our results showed that *S.typhi* isolated from chicken was resistant to Ampicillin, In regard to *S.aureus* antibiotic resistance pattern was inagreement to [13] since they obtain high proportion of Methicillin resistant *S.aureus* MRSA isolates and multidrug resistant strains of *S.aureus*.

Table 3- Diameter in (mm) of inhibition zone of selected antibiotics against *S.aureus*

Descriptive Statistics			
Dependent Variable: Inhibition zone			
antibiotic	Mean	Std. Deviation	N
NA	21.00	1.414	2
CN	14.00	1.414	2
K	20.00	2.828	2
Total	18.33	3.724	6

Table 4- Tests of Between-Subjects Effects

Dependent Variable: Inhibition zone					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	2074.000 ^a	3	691.333	172.833	.001
antibiotic	2074.000	3	691.333	172.833	.001
Error	12.000	3	4.000		
Total	2086.000	6			

a. R Squared = .994 (Adjusted R Squared = .988)

Table 5-Multiple Comparisons

Dependent Variable: Inhibition zone						
LSD						
(I) antibiotic	(J) antibiotic	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NA	CN	7.00*	2.000	.039	.64	13.36
	K	1.00	2.000	.651	-5.36	7.36
CN	NA	-7.00*	2.000	.039	-13.36	-.64
	K	-6.00	2.000	.058	-12.36	.36
K	NA	-1.00	2.000	.651	-7.36	5.36
	CN	6.00	2.000	.058	-.36	12.36

Based on observed means.

The error term is Mean Square (Error) = 4.000.

*. The mean difference is significant at the 0.05 level.

Table 6- Diameter in (mm) of inhibition zone of selected antibiotics against *S.typhi*

Descriptive Statistics			
Dependent Variable: Inhibition zone			
antibiotic	Mean	Std. Deviation	N
c	22.00	2.828	2
TE	25.00	1.414	2
S	13.00	1.414	2
Total	20.00	5.797	6

Table 7-Tests of Between-Subjects Effects

Dependent Variable: Inhibition zone					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	2556.000 ^a	3	852.000	213.000	.001
antibiotic	2556.000	3	852.000	213.000	.001
Error	12.000	3	4.000		
Total	2568.000	6			

a. R Squared = .995 (Adjusted R Squared = .991)

Table 8-Multiple Comparisons

Dependent Variable: Inhibition zone						
LSD						
(I) antibiotic	(J) antibiotic	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
c	TE	-3.00	2.000	.231	-9.36	3.36
	S	9.00*	2.000	.020	2.64	15.36
TE	c	3.00	2.000	.231	-3.36	9.36
	S	12.00*	2.000	.009	5.64	18.36
S	c	-9.00*	2.000	.020	-15.36	-2.64
	TE	-12.00*	2.000	.009	-18.36	-5.64

Based on observed means.

The error term is Mean Square (Error) = 4.000.

*. The mean difference is significant at the 0.05 level.

This is well established facts that contaminated food is main source of transmission for pathogenic bacteria. It is the major cause of enteric disease in developing countries and is a major cause of mortality and morbidity. Hash meat and poultry meats as main source of food borne infection have great impact in food safety .In our study the presence of *S.typhi* and *S.aureus* in local hash meat and poultry meat with some drug resistance isolates is a great public health concern. It is revealed in our study that the presence of *S.typhi* and *S.aureus* is potential threat to consumer health and the development of drug resistance by these common pathogens is amatter of concern in food safety. Thus we search a novel bacteriocin that is necessary for the treatment of multidrug resistant bacteria.

Conclusions

Our study confirmed the presence of *S.aureus* & *S.typhi* in hash meat & poultry meat, it is a potential threat to consumer health, to reduce the risk of contamination, good hygiene practices are necessary from processing to storage. Also crud bacteriocin may use as food preservative.

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