Probiotic Activity of Lactobacillus spp. from Vaginal Specimens against Bacterial Pathogens

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Abstract

Urogenital infections affect millions of people every year worldwide. The management of these diseases usually requires the use of antimicrobial agents. And more newly, the use of probiotic Lactic acid bacteria [LAB] cultures in the management of vaginal infections and other infections has been extensively studied. In this work 30 isolates of *Lactobacillus spp*. Were obtained from healthful Iraqwomen's vagina. All the isolates were subjected to the cultural microscopically and biochemical examinations for the identification of species add to the identification by Vitek2 system [ANC card]. The results showed that half of isolates belongs to *Lactobacillus casei* and the other half belongs to *Lactobacillus gasseri*. agar well diffusion method and disc method are used to detect of bacteriocin production by*Lactobacillus.casei* isolates against 4 species of Gram positive and Gram negative pathogenic bacteria which included:*Corynebacteriumurealyticum*, *Staphylococcus aureus* , *Escherichia coli* and *Psudomonasaeuroginosa*, which obtained from IMAMEIN KADHIMEN MEDICAL CITY . The results showed the Lactobacillus which isolated from vagina by well diffusion method was effective against pathogenic isolates more than the *Lactobacillus* isolated by blank disc method , the high inhibitory effect of *Lactobacillus* isolates by well give an inhibition zone reached to [26] mm , while the Lactobacillus by disc was lower with inhibition reached to [18]mm. The supernatant did not show any activity when was treated with NaOH and adjusted to pH 7. This indicates that the organic acid produced by the *Lactobacillus*isolates was may be actually responsible for the inhibition of the indicator bacteria .The result show that the *Lactobacillus*strains could be considered as potential antimicrobial probiotic strains against some human vaginal pathogens and should be further studied for their human health benefits .

Keywords: bacterial pathogens, lac obacillus ssp.

فعالية المعززات الحيوية لبكتريا حامض اللاكتيك المعزولة من العينات المهبلية ضد الممرضات البكتيرية

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الخلاصة

التهابات المجاري البولية تؤثر على الملايين الناس كل عام في جميع انحاء العالم, ان علاج الامراض عادة يتطلب استخدام عوامل مضادة للمكروبات, حديثا تم استخدام المعززات الحيوية لبكتريا حامض اللاكتيك لعلاج الاصابات المهبلية و الاصابات الاخرى, تمت الدراسة (٣٠) عزلة من بكتريا حامض اللاكتيك وتم الحصول عليها من مهبل النساء العراقيات السليمات و خضعت

جميع العزلات للاختبارات المجهريهوالزرعيه و البايوكيمياويةلغرض معرفة الانواع اضافه الى التشخيص بواسطة نظام الفايتك, النتاىج بينت ان نصف العزلات تابعه الى نوع الاول. استخدمت طريقة الانتشار بالحفر و طريقة الاقراص لغرض الكشف عن العزلات المنتجة للبكتريوسين والمستخدم لاختبار فعالية التثبيطية ضد اربعة انواع من البكتريا الموجبة والسالبة لصبغة كرام والتي تشمل [*Corynebacteriumurealyticum*, *Staphylococcus aureus*, *Escherichia coli* and *Psudomonasaeuroginosa*] وتم الحصول عليها من مختبرات مستشفى مدينة الامامين الكاظمين.ع. النتاىج اظهرت ان عزلات بكتريا حامض اللاكتيك بواسطة طريقة الانتشار بالحفر تاثيرها على عزلات البكتريا المرضية اكثر فعالية من طريقة الاقراص حيث يصل اعلى قطر لمنطقة التثبيط الى (٢٦) ملم بينما اعلى قطر لمناطق التثبيط بطريقة الاقراص يصل الى (١٨) ملم .بينت النتاءج قابلية بكتريا حامض اللاكتيك على تثبيط البكتريا المرضية المسببه للالتهابات المهبلية و الاستفادة منها في علاج مثل تلك الحالات.

الكلمات المفتاحية: الممرضات البكتيرية، حامض اللاكتيك.

Introduction

The vaginalmicroflorawas first reported by Albert Döderlein, as early as 1892.Döderlein found that the micro flora wereregular colonizedWith Gram-positive rods, which remained designated the name "Döderlein'sbacilli".Over the years, these bacillus have been identified as *Lactobacillus*spp. *Lactobacilli*, the predominant micro-organismsof the vaginal macrobiotics [1]. *Lactobacilli* are facultative anaerobic, non-spore forming, catalase negative, rod-shaped lactic acid bacteria., Numerous strains of the genus Lactobacillus are use as probiotics [2]. *Lactobacillus* play a main role in the preservation of a healthy genital tract by avoiding the colonization of pathogenic bacteria, In the healthy women, the vaginal micro flora is ordered by *Lactobacillus* species, at a level of 10^7-10^8 CFU g⁄1 of fluid, which exert a significant effect on the micro flora of the ecosystem, [2]. It has been perceived that indigenous *lactobacilli* prevent the overgrowth and invasion of pathogenic bacteria by a combination of competitive prohibition, competition for nutrients, and release of antimicrobial materials such as hydrogen peroxide, organic acids, bacteriocins, and bio surfactants[3]. In result, a depletion of vaginal lactobacilli has been directlyconnected with an increase in the incidence of genital and urinary infections[4] . The *Lactobacilli* have been shown to produce bacteriocis and collagen binding proteins that prevent pathogen adhesion and displace the pathogens[5]. Probiotics for animals are defined as live microbe that are capable to decrease the number of intestinal infections, increase production and develop food hygiene by contributing to an enhanced gastrointestinal environment [6]. This study was aimed to; study was planned to identify the most common of pathogenic bacteria in the vagina in Iraqi women and estimate the antagonistic effects of *Lactobacillus*that isolated from vaginal tract on the growth of these bacterial isolates including:*Staphylococcus aureus*, *Escherichiacoli*,*Corynebacteriumurealyticum* and *Pseudomonas aeruginosa*.

Materials and methods

Bacteria and cultural conditions

Three pathogenic bacteria were used in the study: *Corynebacteriumurealyticum* , *Staphylococcus aureus* , *Escherichia coli and Pseudomonas aeuroginosa*. These strains were

isolated from different infections and were identified by using conventional method and vitek 2.

Lactobacillus spp.Vaginal samples we recollected from vaginal wall of women and were inoculated on de Man, Rogosa and Sharpe agar medium (MRS) and incubated overnight anaerobically (anaerobic jar and gas pack) at 37 ºC for 48h.growth was streaked on MRS agar plates several times. The isolates were identified to genus level by: gram staining, oxidase, catalase and by the Vitek2 system [ANC card] . The *Lactobacillus* isolates were maintained in MRS broth with 20% glycerol at – 18 ºC as stock culture.

Detection of Bacteriocin Production

1.Well diffusion technique

Bacteria were designated for their antibacterial activity by agar –well diffusion method, the isolates were grown in MRS broth anaerobically at 37 ºC for 48hours.cell free solution were prepared by centrifugation of grown cultures (6000 rpm for 15 min. at 4 ºC)shadowed by filtration using 0.20µm pore size filter ,and obtained supernatants. Brain heart infusion broth medium(BHI) was seeded with overnight culture of *C.urealyticum , S.aureusa , E.coli* and *Ps.aeuroginosa*ending concentration 10^6 cell/ ml, poured into sterile petri dishes and permitted to solidify at room temperature, 6mm diameters well that has been cut in Mueller Hinton agar plates and spotted on with the pathogenic bacteria , the wells filled with 50µl of sterile supernatant separately and allowed to diffuse into agar for 6 hrs at 4 ºC . After (18-24) hours of incubation, the diameters of the zones of growing inhibition were measured. The screening of the antibacterial substances was achieved by using the agar spot test and the well diffusion method defined by [7] was used the growth inhibition presented a clear zone around the tested colonies.

2.Disk technique

Bacteria were designated for antibacterial activity by disk method,. The isolates were grown in MRS broth anaerobically at 37 ºC for 48hours, free cell were prepared by, centrifugation of grown cultures 6000 rpm, for 15 min. at 4 ºC. ,followed by filtration with 0.20µm pore size filter ,and obtained supernatants. Overnight culture of *C.urealyticum , S.aureus , E.coli and Ps.aeuroginosa*at final concentration 10^6 cell/ ml,that culture preub on BHI was poured into sterile petri dishes and allowed to solidify at room temperature, in Mueller Hinton agar plates and spotted on with the pathogenic bacteria , A cork borer 5mm diameter , was used to withdraw disks of filter paper and put in sterile *Lactobacillus* supernatant and forceps was used to place the disks on the surface of the agar, all antimicrobial disk one at a time[8].

Production of Crude Bacteriocin

Supernatant solutions were acquired by growing the inhibitory producer strains overnight in MRS or M17 broth. After incubation at 37°C over 18 to 24 hrs, the cultures were centrifuged and the cell-free supernatant recovered and separated into aliquots that were untreated (crude extract), lyophilized, precipitated with ammonium sulfate subjected to

adsorption-desorption process as described by[9].For the first process, 50 ml aliquots of cell-free cultures were lyophilized (freezing step at -15°C during 24 h; sublimation step for 24 h) and suspended in 5 ml of distilled water (LS supernatant). The ammonium sulfate precipitation of cell-free supernatants was performed as follow: a size of 50 ml of culture supernatant were made up to 60% saturation by addition of ammonium sulfate and kept overnight at 4°C with gentle stirring. After centrifugation (10,000 × g, 20 min, 4°C), the sediment pellet was recovered and suspended in 3 ml of 0.1 M phosphate buffer saline [pbs] at pH6 . For adsorption-desorption method, a 100 ml of supernatant culture was used and its pH adjusted at 6.5to permit adsorption of the bacteriocin to the wall of the producer cell. Then, a temperature of 70°C for 30 min was applied to the culture to kill cells and to inactivate proteolytic enzymes. Cells were then removed by centrifugation at 10,000 × g 20 min, 4°C, and washed twice with 5 mm phosphate buffer saline at pH 6.5. Cell precipitates were suspended in 5 ml of 100 mm NaCl solution adjusted to pH 2 for permitting desorption of the bacteriocin. Stirring was applied for 2 hour at 4°C and the supernatant (ADS) was recovered after centrifugation at 18,000 × g (30 min, 4°C).

To exclude inhibitory special effects of hydrogen peroxide or organic acids, the cell-free extract solutions were dialyzed overnight at 4°C by a dialysis membrane with a 3.5Dalton cutoff against 1.0 liter of distilled water with two changes of distilled water,. After dialysis, the solution in the dialysis bag was filter-sterilized 0.2 μm pore-size filter or heated 70°C, 20 min. Samples were stored at -15°C until use., spots The cell-free extracts were tested for bacteriocin activity against indicator bacteria by using agar diffusion techniques (agar spot test or agar well test). The agar spot method was performed as follows: a fraction of 0.1 ml of an overnight culture of indicator bacteria was poured onto an appropriate medium agar plate. Then,., one drop of all supernatant fluid with antibacterial activity was spotted on the plate. After incubation for 24hrs at temperatures optimal for the indicator bacteria, inhibition was indicated by a clear zone around [9] [10] . positive and negative controls were dispensed plates were incubated overnight at optimal temperature through 24 h. in wells and Inhibition of growth was determined by azone of inhibition surrounding each agar well[11].

Results and Discussion

Identification of pathogenic bacteria

Four pathogenic bacteria were used in the study *C.urealyticum , S.aureus , E.coli and Ps.aeuroginosa,* these strains were isolated from different infections , were identified by using convertional methods by the IMAMEN KADHIMEN MEDICAL CITY, add to the identified by the vitek2 system(Table 1).

Site of Isolation the pathogenic bacteria.Tabla(1):

Pathogenic bacteria	Site of Isolation
C.urealyticum	Genital tract [vaginal swab]
S.aureus	Blood
E.coli	Urinary tract infection [UTI]
Ps.aeuroginosa	Otitis media

Isolation and Identification of Lactobacillus Spp. Vaginal samples were obtained in order to isolate. (30) isolates belong to Lactobacillus genus were obtained depending on the cultural, micoscopic examination and biochemical tests and Vitek2 system.

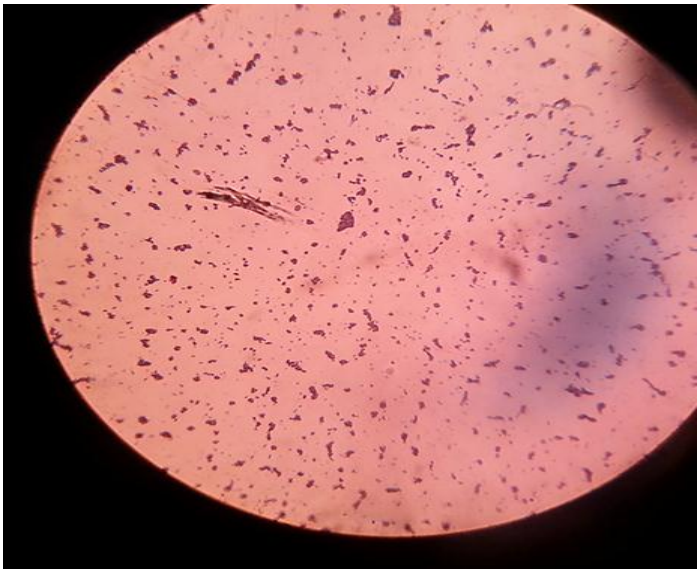

Figure (3-1): *Lactobacillus*appears single, in pairs or in shortchains purple rod cellsisolated from Iraqi women vagina.

Biochemical Tests of *Lactobacillus spp*.

The biochemical characteristics of the *Lactobacillus spp*isolates were similar.All the bacterial isolates were catalase, oxidase negative, unable to grow at 15°C while they were able to grow at 45°C. all *Lactobacillus* isolates were able to grow in the presence of (6.5 and 7) % NaCl whereas at 10% NaCl they were not able to grow.

According to the morphological and biochemical tests, the isolates were identified as *Lactobacillus* spp. Depending on the results of vitek2 system . Were used the vitek2 system for identification bacterial isolated that isolate from healthful women vagina to species ,the results when used specific ANC card for gram positive bacterial species obtainment the half isolate is *Lactobacillus casei* and the other is *Lactobacillus gasseri* .*Table (2)*

Table (2): Biochemical test of Lactobacillus spp identification by vitek2 system

Test	Result
dGAL	**+**
dCEL	**+**
SAC	**-**
BGALI	**-**
MTE	**+**
PHOS	**-**
GRAM	**+**
LeuA	**+**

TYrA	**+**
ARB	**+**
AARA	**-**
ESC	**+**
IARA	**-**
MORPH	**-**
ELLM	**-**
APPA	**+**
NAG	**+**
AGALi	**-**
BdFUC	**-**
dRIB2	**+**
AERO	**-**
PheA	**+**
dGLU	**+**
BGLUi	**+**
BMAN	**-**
BNAGi	**-**
OPS	**-**
ProA	**+**
dMNE	**+**
URE	**-**
ARG	**-**
AMANi	**-**
AARAF	**-**
PyrA	**+**
dMAL	**+**
BGURi	**-**
PVATE	**-**
AIFUC	**-**
Dxyl	**-**

+ :Positive result

-:Nagitive result

The biochemical tests of vitek2 system is include;(Ala-Phe-Pro-ARYLAMIDASE , L-PYrrolydonyl-ARYLAMIDASE , D-CELLOBIOSE , D-GLUOSE , BETA-GLUCOSIDASE, D-MALTOSE , D-MANNOSE , L-POLINE ARYLAMIDASE , Tyrosine ARYLAMIDASE , MALTOTRIOSE , Leucine ARYLAMIDASE , Phenylalanine ARYLAMIDASE , ARBUTIN , N-ACETYL-D-GLUCOSAMINE and ESCULIN hydrolysis) is positive tests while the negative tests included; (BETA-GALACTOSIDASE , BETA-N-ACETYL-GLUCOSAMINIDASE , UREASE , SACCHAROSE/SUCROSE , ALPHA-GALACTOSIDASE , PHOSPHATASE , D-XYLOSE , ALPHA-L-ARABINOFURANOSIDE , Phenyl phosphonate , ALPhA-L-FUCOSIDASE , D-GALACTOSE , ELLMAN , ALPHA-ARABINOSIDASE and BETA-D-FUCOSIDASE).

Table3: ANC Offline Tests

Test Name	Test	Result	Definition
AERO	**Aerotolerance**	**-**	**Anaerobe**
MORPH	**Morphology**	**-**	**Bacilli**

Table 4:Identification Information of Lactobacillus spp. By the (ANC CARD) Vitek2 system.

Species of organism	Bionumber	Probability
Lactobacillus gasseri	**2777610030401**	**90**
Lactobacillus casei	**3377630020001**	**90**

Detection of antagonistic activity of isolated cultures
Wells method In liquid media

The Inhibitory effect of *Lactobacillus*isolates grown in MRS broth was evaluated also, . The Well diffusiontechnique was used to determine the inhibitoryaction of *Lactobacillus*against pathogenic isolates. The highs inhibitory effect was achieved when using supernatant of *Lactobacillus*[8].Only 18 isolates of *Lactobacillus spp*presented high antibacterial activity against indicator bacteria , 2 isolates of Gram positive bacteria [*Corynebacteriumurelyticum, Staphylococcus aureus*] and 2 isolates of Gram negative bacteria [*Escherichia coli* , *Pseudomonas aeruginosa*] crude filtrate supernatant solutions of *Lactobacillus spp* isolates showed antibacterial activity against indicator bacteria with inhibition zone diameters between 6-27mm as shown in table (5).

Table (5): Detection of bacteriocin production from Lactobacillus spp. isolates by agar well diffusion assay against indicator bacteria
Number ; diameter of inhibition zone
no inhibition: -

Lactobacillus spp.	***S.aureus***	***C.urealyticum***	***Ps.aeuroginosa***	***E.coli***
Lb.1	**18**	**16**	**-**	**-**
Lb.2	**12**	**22**	**-**	**-**
Lb.3	**-**	**-**	**-**	**-**
Lb.4	**8**	**12**	**-**	**-**
Lb.5	**-**	**-**	**-**	**-**
Lb.6	**20**	**18**	**-**	**-**
Lb.7	**-**	**-**	**-**	**-**
Lb.8	**19**	**14**	**-**	**-**
Lb.9	**-**	**-**	**-**	**-**

Lb.10	**16**	**22**	**-**	**-**
Lb.11	**12**	**-**	**-**	**-**
Lb.12	**10**	**14**	**-**	**-**
Lb.13	**16**	**17**	**-**	**-**
Lb.14	**20**	**23**	**12**	**-**
Lb.15	**8**	**10**	**-**	**-**
Lb.16	**-**	**-**	**-**	**-**
Lb.17	**19**	**16**	**-**	**-**
Lb.18	**25**	**21**	**-**	**-**
Lb.19	**16**	**18**	**-**	**-**
Lb.20	**22**	**25**	**-**	**14**
Lb.21	**26**	**24**	**8**	**6**
Lb.22	**15**	**12**	**-**	**-**
Lb.23	**24**	**23**	**-**	**11**
Lb.24	**18**	**20**	**-**	**-**
Lb.25	**16**	**12**	**-**	**-**
Lb.26	**-**	**-**	**-**	**-**
Lb.27	**12**	**16**	**-**	**-**
Lb.28	**14**	**10**	**-**	**-**
Lb.29	**-**	**-**	**-**	**-**
Lb.30	**20**	**18**	**-**	**-**

Discmethod on solid media

The results revealed that proliferation of *Lactobacillus* isolates on MRS agar under anaerobic condition was an effective method for the production of their inhibitory metabolites against tested pathogens. In this approach [8] [7],start that using MRS agar medium in studying the ability of *Lactobacillus* isolates to produce inhibiting materials under anaerobic condition,. in the selected procedure that gives reasonable result. the results showed the *Lactobacillus* which isolated from vagina by well diffusion method was effective against pathogenic isolates more than the *Lactobacillus* isolated by blank disc method .The high inhibitory result of *Lactobacillus* isolates by well give an inhibition zone reached to 26 mm while the *Lactobacillus* by disc was lower with inhibition zone reached to 18mm the Table (6) shows *Lactobacillus spp* isolated from vagina on solid MRS media by disc method, in the chosen procedure that gives reasonable result.

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Table(6):**Detection of bacteriocin production from *Lactobacillus spp*. Isolates by disc method against indicator bacteria** .

Lactobacillus spp.	***S.aureus***	***C.urealyticum***	***Ps.aeuroginosa***	***E.coli***
Lb.1	**9.5**	**1O**	**-**	**-**
Lb.2	**9**	**12**	**-**	**-**
Lb.3	**-**	**-**	**-**	**-**
Lb.4	**-**	**8**	**-**	**-**
Lb.5	**-**	**-**	**-**	**-**
Lb.6	**12**	**14.5**	**-**	**-**

Lb.7	**-**	**-**	**-**	**-**
Lb.8	**7**	**10.6**	**-**	**-**
Lb.9	**-**	**-**	**-**	**-**
Lb.10	**6.5**	**11**	**-**	**-**
Lb.11	**8**	**6**	**-**	**-**
Lb.12	**5.5**	**6**	**-**	**-**
Lb.13	**11**	**14**	**-**	**-**
Lb.14	**18**	**12.5**	**-**	**-**
Lb.15	**4.4**	**7**	**-**	**-**
Lb.16	**-**	**-**	**-**	**-**
Lb.17	**16**	**14.5**	**-**	**-**
Lb.18	**10**	**17**	**-**	**-**
Lb.19	**8.6**	**12**	**-**	**-**
Lb.20	**14**	**18**	**-**	**-**
Lb.21	**16.5**	**16**	**-**	**-**
Lb.22	**5.5**	**9**	**-**	**-**
Lb.23	**12.5**	**10**	**-**	**-**
Lb.24	**8**	**11.4**	**-**	**-**
Lb.25	**5**	**9.5**	**-**	**-**
Lb.26	**-**	**-**	**-**	**-**
Lb.27	**6.6**	**11**	**-**	**-**
Lb.28	**8**	**5**	**-**	**-**
Lb.29	**-**	**-**	**-**	**-**
Lb.30	**15**	**12.5**	**-**	**-**

Were the results above display some bacterial isolates of *Lb.spp* able to inhibit almost all the indicator bacteria , while others were active against only few isolates. Also, only a few of *Lb.spp* isolates that testedpositive using well diffusion method gave positive results in the other method .Suggesting that the discovery of production of bacteriocin in broth medium was best than in solid medium as previously testified for some bacteriocins [12].

A simply explanation for this observation is that bacteriocins probable adsorption at the cell surface of producer, whereas they are diffusion in the whole medium of liquefied cultures as investigated by[13] (Hindre et al., 2003) However, the well diffusion test has the advantage of permitting the bacteriocin to diffuse into the agar before the indicator strains initiated to grow[14].

Extraction of bacteriocin with Ammonium sulphate

The partial purification of bacteriocin was accomplished by extraction with ammonium sulfate ,the ammonium sulfate precipitation of cell-free supernatants was achieved as follow: a volume of 50 ml of culture supernatant were made up to 60% saturation by addition of ammonium sulfate and kept overnight at 4°C with gentle stirring.the sediment pellet wasrecovered and suspended in 3 ml of 0.1 M phosphate buffer saline [pbs] at pH6 , For [adsorption-desorption technique]portentous that at least part of the molecule has a hydrophobic character and bonds this property with other bacteriocins[15] [16] [17].The

antibacterial activity of crude bacteriocin on the pathogenic bacteria include; 2 isolates of Gram positive bacteria [*Staphylococcusaureus , Corynebacteriumurealyticum*] and 2 isolates of Gram negative bacteria [*Escherichia coli* , *Pseudomonas aeruginosa*]. The results indicated that crude bacteriocin of *L.casei* possessed significant high antibacterial activity against all *C.urealyticum* and *S.aureus* isolates of Gram positive bacterial group while the antibacterial activity of crude bacteriocin was less or no affect against *E.coli* and *Ps.aeruginosa*isolates of Gram negative bacterial group contrast with control,In this study Crude bacteriocin recorded maximum antibacterial activity against *C.urealyticum*(28,20)mm and *S.aureus*(21.5 ,18)mm. [18] reported that, the bactericidal or bacteriostatic actioncontrolled by bacteriocins is partial by the following factors: bacteriocin dose and purification amount, physiological status of the indicator cells (e.g. growth phase) and experimental conditions (e.g. , pH ,temperature., presence of agents disrupting cell wall integrity and other antimicrobial compounds). Other bacteriocins with bactericidal type of action without cell lysis have also beenreported[19] [20] [21] [22].

Moreover, it has to be stated that the antibacterial type of action of bacteriocins appears to be dependent on several factors such as the concentration and purity of the bacteriocin preparation, the type of buffer or broth used, the sensitivity of the indicator strain tested and the density of the cell suspension applied[23].In overall the bactericidal/bacteriostatic action of bacteriocins involves the increased permeability of the cytoplasmic membrane of the target cells for a widespread range of monovalent cations (e.g: K+, Li+, Cs+,Rb+,Na+ and choline) principal to the destruction of proton motive force by dissipation of the transmembrane pH valuation and eventually to the cell death [24] [25] Bacteriocins produced by Lactic acid bacteria showed the results against indicator strains, gave the maximum level of activity against *L. monocytogenes*[26], which showed inhibitory efficiency by the targeting of cytoplasmic membrane[27] [28].

Table 7:The antibacterial activity of crude bacteriocin on the Pathogenic bacteria.

Crude bacteriocin	***C.urealyticum***	***S.aureus***	***E.coli***	***Ps.aeruginosa***
Concentrate	28mm 20mm	21.5mm 18mm	-	-

Conclusions

Lactobacillus casei has been found to dominate among *Lactobacillusspp* in healthful women vagina samples.The ability to produce bacteriocin seems to be scarce among *Lb.casei* isolated from vagina ,and The agar well diffusion assay and disc method was considered the sufficient fordetection of bacteriocin production , indicating that production of bacteriocin was best in broth medium comparison to the solid medium,The highest activity was against *C.urealyticum* and *S.aureus* while no activity observed against *E.coli* and *Ps.aeruginosa* . The best producer isolate was *Lb.casei* which produced it in broth media [MRS] used in this study.

References

[1] **Boris**,S. and Barbes,C. **2000**. Role played by *lactobacilli* in controlling the population of vaginal pathogens. Microbes.Infect., 2:543-546.

[2] **Walencka**, E.;Rozalska, S.;Sadowska, B. and Rozalska, B. **2008**.The influence of *Lactobacillus acidophilus*–derived surfactants on *staphylococcal* adhesion and biofilm formation.FoliaMicrobiologica53:61-66.

[3] **Aroutcheva**, A.; Dominique, G.; Melissa, S. and Susan, S.**2001**.Defence factors of vaginal *lactobacilli*.American Journal of Obstet. and Gynocol.,185(2):375-379.

[4] **Pavlova**, S.I.;Kilic, A.O. and Kilic, S.S. **2002**. Genetic diversity of vaginal *lactobacilli* fromwomen in different countries based on 16S rRNA gene sequences. J. Appl.Microb.,92(3):451–459.

[5] **Jassawala**, M.J.**2005**. Probiotics and Womensheath.J.Obstet Gynecol. India, 54(1):19-21.

[6] **Nousiainen**, J.;Javanainen, P.;Setala, J. and von Wright, A.**2004**.*Lactic acid* bacteria as animal probiotics. In:Salminen S, Ouwehand A, von Wright A (eds.): *Lactic Acid* Bacteria: Microbial and Functional Aspects. 3rd ed. Marcel Dekker, New York.

[7] **Vignolo**, G.; Palacios, J.; Farias ,M.E.; Sesma, F.; Schilling ,U .; Holzapfel ,W.and Oliver, G.**2000**. Combind effect of bacteriocins on the survival of listeria species in broth and meat system .Curr.Microbiol. 41(6), 410-416.

[8] **AL-Kafaji** , Z. M**. 1992** . Influence of various condation on Inhibitory effect of intestinal *Lactobacillus*against intestinal bacteria causes diarrhea. Iraqi J. of Science,3(1):18-26 .

[9] **Yang**, R., Johnson, M.C. & Ray, B. 1992. Novel method to extract large amounts of bacteriocins from *lactic acid* bacteria. Appl. Environ. Microbiol. 58(10), 3355–3359.

[10] **Cintas**, L.M., Casaus M.P., Holo H., Hernandez P.E., Nes, I.F. &Havarstein, L.S. 1998. Enterocins L50A and L50B, two novelbacteriocins from *Enterococcus faecium* L50, are related to *Staphylococcal*hemolysins. J. Bacteriol. **180**(8): 1988‑1994.

[11] **Herranz**, C., Casaus P., Mukhopadhyay S., Martinez J.M., Rodriguez J.M., Nes I.F., Hernandez P. E. & L. M. Cintas. 2001. Enterococcus faeciumP21: a strain occurring naturally in dryfermented sausages producing the Class II bacteriocinenterocin A and enterocin B. Food Microbiol. **18**, 115‑131.

[12] **Leroy**, F. and De Vuyst, L. (1999) Temperature and pH conditions that prevail during fermentation of sausages are optimal for production of the antilisterialbacteriocinsakacin K. Appl. Environ. Microbiol., 65(3): 974 –981.

[13] **Hindre**, T.; Didelot, S.; Pennec, J.P.L.; Haras, D.; Dufour, A. and Vallée – Réhel, K. (2003) Bacteriocin detection from whole bacteria by matrix – assisted laser desorption ionization – time of flight mass spectrometry. Appl. Environ. Microbiol., 69(2): 1051 – 1058.

[14] **Tagg**, J.R. and McGiven, A.R.(1971) Assay system for bacteriocins.Appl. Microbiol., 21(5):943.

[15] **Daba**, H.; Pandiau, S.; Gosselin, J.F.; Simard, R.E.; Huang, J. and Lacroix, C. (1991). Detection and activity of a bacteriocin produced by Leuconostocmesenteroides. Appl. Environ. Microbiol., 57 (12):3450-3455.

[16] **Enan**, G.(2006b). Nature and phenotypic characterization of plantaricin UG1 resistance in Listeria monocytogenes LMG 10470. J.Food .Agricul. .Environ, 4(1):105-108.

[17] **Noonpakdee**, W.; Jumriangrit, P.; Wittayakom, K.; Zendo, J.; Nakayama, J.; Sonomoto, K. and Panyim, S. (2009). Two – peptide bacteriocin from *Lactobacillus plantarum* PMU 33 strain isolated from som – fak, a Thai low salt fermented fish product. Asia Pacific J. Mol. Biol. Biotechnol., 17(1): 19 – 25.

[18] **Deraz**, S.F. Karlsson, E.N., Khalil, A.A., Mattiasson, B. (2007). Mode of action of acidocin D20079, a bacteriocin produced by the potential probiotic strain, *Lactobacillus acidophilus* DSM 20079.J .Ind .Microbiol .Biotechnol. May;34(5):373-9.

[19] **Garriga**, M.;Hugas, M.;Aymerich, T. and Monfort, J.M.(1993). Bacteriocinogenic activity of *lactobacilli* from fermented sausages.J.Appl. Bacteriol., (75):142-148.

[20] **Kelly**, W.J.; Asmundson, R.V. and Huang, C.M. (1996) .Characterization of plantaricin KW30, a bacteriocin produced by Lactobacillus plantarum. J. Appl. Bacteriol.,(81): 657 – 662.

[21] **Enan**, G.; El – Essawy, A.A.; Uyttendaele, M. and Debevere, J. (1996) .Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. Int.J.Food .Microbiol., (30): 189 – 215.

[22] **Messi**, P.; Bondi, M.; Sabia, C.; Battini, R. and Manicardi, G. (2001) .Detection and preliminary characterization of a bacteriocin (plantaricin 35d) produced by a *Lactobacillus plantarum* strain. Int. J. Food. Microbiol., (64): 193 – 198.

[23] **Messens**, W. and De Vuyst, L. (2002). Inhibitory substances produced by *lactobacilli* isolated from sourdoughs – a review. Int. J. Food. Microbiol., (72): 31 – 43.

[24] **Oppegerd**, C.; Fimland, G., Thorbok, L., Nissen-Meyer, J. (2007). Analysis of the two-peptide bacteriocinsLactococcin G and Enterocin 1071 by site-directed mutagenesis. Appl .Environ.Microbiol, 73 (9): 2931–2938.

[25] **Simova**, E.D.; Beshkova, D.B.; Dimitrov, Z.P. (2009). Characterization and antimicrobial spectrum of bacteriocins produced by lactic acid bacteria isolated from traditional Bulgarian dairy products. J. Appl. Microbiol. 106 (2):692–701.

[26] **Ibarguren**, C.; Raya, R.R.; Apella, M.C.; Audisio, M.C. (2010). *Enterococcus faecium* isolated from honey synthesized bacteriocin-like substances active against different Listeria monocytogenes strains. J. Microbiol. Seoul Korea, 48: 44 52.

[27] **Delves**-Broughton, J., Blackburn, P., Evans, R.J., Hugenholtz, J. (1996). applications of the bacteriocin, nisin. Antonie Van Leeuwenhoek, (69): 193 202.

[28] **Gálvez**, A.; Abriouel, H.; López, R.L.; Ben Omar, N. (2007). Bacteriocin-based strategies for food biopreservation. Int. J. Food .Microbiol., (120): 51 70.