# Antimicrobial Effect of Bacteriocin produced *Pediococcus pentosaceus* on some clinical isolates

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## ABSTRACT

About 10 isolates of ***Pediococcus*** sp were isolated from different cheese made in Iraq, These isolates were identified morphologically and biochemically and Api20 kit, thus there was only 6 isolate were identified as ***Pediococcus pentosaceus*** (60%).In this study, we investigate, the effect of crude Bacteriocin from ***Pediococcus pentosaceus*** on 30 clinical isolates (5 ***E.coli***, 5 ***Klepsiella pneumoniae***, 5 ***Staphylococcus aureus***, 5 ***Pseudomonas aeroginosa***, *5* ***Bacillus subtilis***, 5 ***Candida albicans***). The protein concentration of this Bacteriocin was measured 67mg\ml by Bradford method and used as (1:2) by vol during the measuring the antimicrobial activity against the above clinical isolates by two methods wells and agar plug assay. The results showed that the inhibitory activity of this Bacteriocin was higher by wells method than agar pluq assay against Gram–positive bacteria or Gram-negative bacteria and yeast under this study.

**Keywords:** Antimicrobial, Bacteriocin**,** Pediococcus pentosaceus**.**

**الخلاصة**

تم عزل (10) عزلات بكتيرية تعود لجنس ***Pediococcus*** من انواع مختلفة من الاجبان المصنوعة في العراق، تم اخضاع هذه العزلات الى فحوصات مظهرية وبايوكيميائية وتشخيصها باستخدام API kit. وقد ظهر وجود 6 عزلات فقط تعود للنوع ***Pentosaceus***اي حوالي (60%).

شملت هذه الدراسة تنقية مادة البكتريوسين من بكتريا ***Pediococcus pentosaceus*** ودراسة تاثيرها على (30) من العزلات السريرية الموجودة في قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية وكانت ***(5 E.coli, 5 Klepsiella pneumoniae, 5 Staph.aureus, 5 Pseudomonas aeroginosa, 5 Bacillus subtilis* and *5 Candida albicans )***

كما تم تحديد تركيز البروتين لهذا المستخلص وكان (76) مايكروغرام/مل بطريقة برادفورد واستخدم بتركيز (2:1) كنسبة حجمية خلال قياس الفعالية ضد المايكروبية ضد العزلات اعلاه بطريقةالحفر wells والقالب Agar pluq assay وقد اظهرت النتائج ان الفعالية ضد المايكروبية لهذا البكتريوسين كانت اعلى باستخدام طريقة الحفر مما هي عليه بطريقة القالب سواء كان ضد مجموعة البكتريا الموجبه او السالبه لصبغة غرام وحتى ضد الخمائر المستخدمه في هذه الدراسة.

## INTRODUCTION

***Pediococcus pentosaceus*** represent member of lactic acid bacteria are coccus shaped microbes, Give non spore forming, non-motile. (1) ***Pediococcus pentosaceus*** are caragterized as lactic acid because the end product of its metabolism is lactic acid. (2) ***Pediococcus pentosaceus*** like most lactic acid bacteria are anaerobic and ferment sugars. Since the end product of metabolism lactic acid ***Pediococcus pentosaceus*** are acid tolerant. They can be found in plant materials, ripened cheese and avariety of processed meats. (3) ***Pediococcus pentosaceus*** is industrially important due to its ability to starter culture to ferment foods such as various meats, vegeTables and cheese. (4) ***Pediococcus pentosaceus*** bacteria is being cultured and researched for its ability to produce antimicrobial agents as well asit is one of most important as preserver. (5) ***Pediococcus pentosaceuscan*** be cultured at 35-40c but are unable to grow at 50c. But able

to grow at pH(4.5-8). (1) This bacteria grow more stably at more acidic PH (4).***Pediococcus pentosaceus*** are unique in that they form tetrads (1). ***Pediococcus pentosaceus*** can produce antimicrobial agents known as bacteriocin (3) agants several species of ***Lactobacillus, Lactococcus, Leuconostic, Pediococcus, Staphylococcus aureus, Bacillus*** and ***Listeria*** (4). The bacteriocin isolated in ***Pediococcus pentosaceus*** was labeled pediocin (4). The end product of fermentation is lactic acid which lowers the environmental pH.

# MATERIALS AND METHODS

### Isolation and Identification of Bacterial Isolates

The present study included (10) Pediococcus samples from different cheeses made in Iraq Bacterial isolates were identified to the species level using the Traditional biochemical and morphological test described by (6) and then confirmed using rapid systems (Api20E) as recommended by the manufacture (Biomeriex-France)

Other Bacterial isolates and yeast were obtained from the college of science, Al-Mustansiriyah University as follow: Gram - positive Bacteria such as *Staphylococcus aureus and Bacillus subtilis.* Gram - negative Bacteria such as *Eschericia coli and Pseudomonas auroginosa, Klebsiella pneumoniae, and* Yeast such as *Candida albicans.*

### Antibiosis

The Bacteriocin production was detected by wells method as described by Gupta *et al.*. (1998) [7] by spreading 0.1ml of tested isolates ($10^8$cfu/ml) (each one alone) on plate of nutrient agar (NA) medium, then the wells prepared & filled with 100$\mu$1 of *Pediococcus Pentosaceus* filterate, the plates incubated at 37ºC for (24)h, then the diameter of inhibition zone were measured in (mm) & compared with the standard plate containing only free bacteria nutrient agar (NA).

### Extraction of Bacteriocin form *Pediococcus pentosaceus*

Depending on the results of Antibiosis the highly *Pediococcus pentosaceus* (4&7) isolutes were used for Bacteriocin extraction by growing at 30ºC on Men Roges Sharp (MRS) liquid medium for (18-24)h. The culture supernatant was then collected and adjusted pH to 6.5, filter through 0.22mm pore size filters, concentrated to 0.1 volume by polyethylene glycol dialysis (sigma PEG20000) and again filter – sterilized. This material was designated crude bacteriocin and was frozen at (-20)ºC when not used immediately [9].

### Estimation of Protein Concentration

Protein concentration was determined by using the dye-binding method[10]. Then the crude protein used as 1:2 by vol to determine the antimicrobial activity.

### The Antimicrobial Activity of Partially Puified Bacteriocin

Two method (wells and plug agar assay) was used for detection of antimicrobial activity of bacteriocin against thirty (30) clinical isolate mention above in two ways:-

### 1- Agar plug assay method [7]

Nutrient agar (NA) plugs containing various concentrations of *Pediococcus* Bacteriocin (0, 125, 250, 500, 1000) mg/ml. These plugs were prepared by Pasteur pipette from a plate with a special concentration. Then the plugs were placed on plates of nutrient agar seeded with a lawn of tested bacterial isolates. or plates of starch yeast extract (SY) with *Candida albicans* yeast.

The plates stored at 4˚C for (20-24) hr, then incubated at 37˚ for further (24) hr. The diameter of inhibition zone was measured in millimeter.

### 2- Wells assay method. [7]

The Bacteriocin was dissolved in sterile distilled water at the following concentrations in mg/ml (0, 125, 250, 500 & 1000), and then 200 ml of each concentration were placed in wells of nutrient agar or (SY) agar plates seeded with tested microorganisms. The plates stored at 4˚C for (20-24) hr, then incubated at 37˚ for further (24) hr. the diameter of inhibition zone was measured in millimeter.

# RESULTS AND DISCUSSION

In this study, we investigated only 6 from 10 isolates of *Pediococcus spp*. which were then

identified morphologically & biochemical, as *Pentococcus pentosaceus*, then the crude Bacteriocin were partially purified according to Piva & Headon [8]. In addition to the protein concentration were detected according to Bradford method by protein dye method, and it was 67 mg/ml [9].

Different concentration from this crude Bacteriocin were used for detected the antimicrobial activity of it against 30 microbial isolates different clinical sources by two methods, wells & agar plug assay as in Table 2. Also, the antibiosis was measured and the results shpwed that the bacteriocin production by *P. pentosacens* was varied among the ten isolates used. and it is highly produced by *P. pentosacens* (4&7) isolate, while the other isolates were lower producers as in Table 1. So. we used only *P. pentosacens* (4&7) for extraction of Bacteriocin.

In this regards, Mehdi *et al.*. (2014) can isolate & purified the Bacteriocin from *Pediococcus spp*. from human source. With yield of (9.08 %) and specific activity was (27540) Au/mg. [10]

## The Antimicrobial Spectrum of partially purified Bacteriocin from *Pediococcus Pentosoceus*

The results of antimicrobial activity of partially purified Bacteriocin were detected by two methods wells & agar plug assay. All the results shows high diameter of inhibition zone by wells method rather than the diameter of inhibition zone by agar plug assay method, against (30) clinical isolates as in Figure 2 and Table 1.

**Table 1:** Results of Antibiosis.

| Inhibi-tion zone in (mm) / *Pediococcus No.* | *S.aureus* | E.coli | *K.pneumoniae* | *Ps.aeroginosa* | *B.subtilis* | *C.alibicans* |
|---|---|---|---|---|---|---|
| 1 | 12 | 14 | 12 | 10 | 11 | 12 |
| 2 | 12 | 12 | 11 | 12 | 11 | 11 |
| 3 | 14 | 12 | 10 | 13 | 10 | 12 |
| 4 | 18 | 16 | 18 | 16 | 16 | 16 |
| 5 | 12 | 11 | 14 | 11 | 10 | 12 |
| 6 | 11 | 14 | 11 | 12 | 11 | 10 |
| 7 | 18 | 17 | 18 | 18 | 16 | 16 |
| 8 | 12 | 12 | 12 | 10 | 12 | 11 |
| 9 | 11 | 10 | 10 | 11 | 12 | 10 |
| 10 | 12 | 11 | 10 | 11 | 12 | 11 |

**Table 2:** the diameter of inhibition zone (in millimeter) of Bacteriocin (1000mg/ml) from *Pediococcus pentosaceus* against 30 Microbial isolates by wells & plug agar method.

| Test microorganisms | Diameter of inhibition zone in (mm) by wells method | Diameter of inhibition zone in (mm) by agar plug assay |
|---|---|---|
| *S. aureus 1* | 40 | 35 |
| *S. aureus 2* | 40 | 32 |
| *S. aureus 3* | Full | 35 |
| *S. aureus 4* | 38 | 33 |
| *S. aureus 5* | 32 | 30 |
| *K. pneumoniae 1* | 35 | 30 |
| *K. pneumoniae 2* | 38 | 32 |
| *K. pneumoniae 3* | 40 | 34 |
| *K. pneumoniae 4* | 35 | 30 |
| *K. pneumoniae 5* | 35 | 30 |
| *P. aeroginosa 1* | 40 | 35 |
| *P. aeroginosa 2* | 38 | 34 |
| *P. aeroginosa 3* | 40 | 30 |
| *P. aeroginosa 4* | 40 | 30 |
| *P. aeroginosa 5* | 40 | 32 |
| *B. subtilis 1* | 35 | 30 |
| *B. subtilis 2* | 32 | 28 |
| *B. subtilis 3* | 30 | 27 |
| *B. subtilis 4* | 28 | 26 |
| *B. subtilis 5* | 30 | 25 |
| *C. alibicans 1* | 30 | 24 |
| *C. alibicans 2* | 28 | 26 |
| *C. alibicans 3* | 28 | 20 |
| *C. alibicans 4* | 30 | 22 |
| *C. alibicans 5* | 28 | 22 |

Our results showed a higher antimicrobial activity of (40) mm to full diameter of inhibition zone against (*S.aureus*) as in Figure (1, 2) and then decrease to 30, 28 by wells method and from (24 to 22 mm) against *Candida albicans*.

This may due to complexity of yeast. Cell structure (metamorphism) specialty of *Candida albicans*, thus it may be more resist to this Bacteriocin in compared with other (25) test microorganisms.

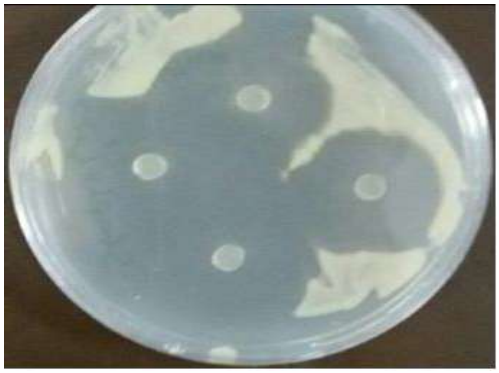

**Figure 1**: The effect of different concentrations of partially purified of Bacteriocin (1000mg/ml) of *Pediococcus pentosaceus* on the diameter of inhibition zone against *S.aureus* measured by agar plug assay method.

**Figure 2**: The effect of different concentrations of partially purified of Bacteriocin (1000mg/ml) of *Pediococcus pentosaceus* on the diameter of inhibition zone against *S.aureus* measured by wells assay method.

Also, the effecte of different concentrations of this Bacteriocin also studied and shown in Figure 3. It shows the higher dimeter of inhibition one (45 mm in dimeter) at Bacteriocin of (1000 mg/ml) concentration, then the diameter will be decrease at (500, 250 mg/ml), while the same diameter (20-30 mm) of inhibition zone at both two (250 & 125 mg/ml) of Bacteriocin concentration.

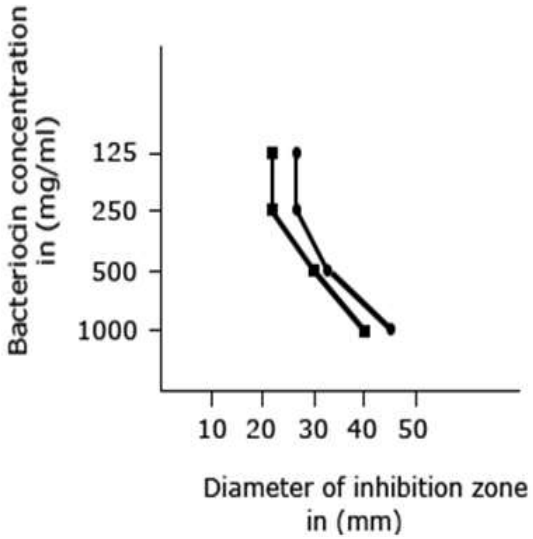


**Figure 3**: The effect of different concentrations of partially purified of Bacteriocin of *Pediococcus pentosaceus* on the diameter of inhibition zone against *S.aureus* measured by wells ( ■ ) & agar plug assay method ( ● ).

These results showed that a high diameter of inhibition zone obtained at (1000 & 500 mg/ml) of Bacteriocin while the same diameter of inhibition zone (28 & 22 mm in diameter) at Bacteriocin of concentration of (250 & 125 mg/ml). And the diameter of inhibition zone was higher in wells method than the agar plug assay method, these results may due to the:

Concentration of Bacteriocin, when it was high a long diameter of inhibition zone will be obtained.

In wells method, the Bacteriocin will diffuse and be more effective than agar plug assay method.

In this regards, there were many studies on Bacteriocin production from *Pediococcus pentosaceus* of of M.wt. 38 KDa after purification, have antifungal activity against *Fusarium graminearum* [12]. Other, can produce Bacteriocin with a M.wt. of 4.8 KDa by *Pediococcus pentosaceus* CFRB 19 [12].

However, productions of Bacteriocin have different characterized from *Pediococcus pentosaceus* with M.wt. 80 KDa [13].

Our results was in agreement with Taher (1998) who found that the antibacterial activity of Actinorhodine – like substance produced by *Streptomses* IQ 45 against strains of *Staph. aureus* was higher in wells method than the agar plug assay method. [14].

Finally, we concluded that this Bacteriocin can be used as food preservatives and show to be

effective in inhabiting the pathogenic and may be food spoilage microorganisms after further purification of this Bacteriocin.

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