TMP UNIVERSAL JOURNAL OF RESEARCH AND REVIEW ARCHIVES

VOLUME 1 | ISSUE 2 | YEAR 2022 | OCT - DEC 2022

RECEIVED DATE	REVISED DATE	ACCEPTED DATE
20/09/2022	18/10/2022	27/11/2022

Article Type: Research Article

Available online: www.tmp.twistingmemoirs.com

ISSN: N/A

PRODUCTION BACTERIOCIN BY *LACTOBACILLUS* SSP AND ANTIBACTERIAL ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* AND BIOFILM FORMATION

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ABSTRACT

Antimicrobial activity of *Lactobacillus spp*. especially (*L. planetarium* and *L. acidophilus*) against *S. aureus* were tested using agar-plug, agar well diffusion methods to select the best isolate that could inhibit the growth of multidrug resistance isolates. Further identification for the presence of bacteriocin was done using ELISA kit. Results showed that *Lactobacillus* spp isolates were bacteriocin producers with different degrees and that *L. planetarium* (*L7*) was the most efficient in bacteriocin production. Therefore, *L. planetarium* (L7) was selected for purification using 70% saturated ammonium sulfate and gel chromatography. The effect of purified bacteriocin was tested on 16 bacterial isolates using micro-titer plate method and well diffusion method. The results showed the ability of the bacteriocin to inhibit bacteria only at concentrations 1866U/ml (50%), 3732U/ml (100%) with a diameter of inhibition zones ranges between (11-23 mm) respectively. The anti-biofilm activity of purified bacteriocin at concentration 100% was investigated and the results showed that biofilm formation was reduced by 100% in the presence of bacteriocin.

Keywords: Bacteriocin, *Lactobacillus*, Antibacterial, *Staphylococcus aureus*, Biofilm

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INTRODUCTION

Staphylococcus aureus is an opportunistic and commensal bacterium can cause infection in diverse parts of the body, such as nostrils, skin, and inguinal areas. Approximately (25-30%) of

healthy persons colonize with *S. aureus* (Feingold *et al.*, 2012). However, up to 80% of people are considering with high risk to colonize with *S. aureus* colonization, including (diabetic persons, health care workers, patients with weak immunity, individuals with long hospital stays, recipients of with previous methicillin- resistant *S. aureus* (MRSA) infection, and skin infections persons). Also, fomite contamination, and food contamination, dialysis patients, surgical operations, indwelling catheter users, individuals with skin infections, individuals with chronic metabolic diseases immune compromised individuals, can consider as a source of infection transmit from one person to another (Habeeb *et al.*, 2014; Tong *et al.*, 2015; Gnanamani *et al.*, 2017). *S. aureus* colonize in diverse parts of the body and the risk increased by infection in the surgical site, in addition to infections and the risk increase by of lower respiratory and blood stream (Gnanamani *et al.*, 2017). *S. aureus* can cause different infections depend on the mechanism and site of occurrence into, Skin and soft tissue infections (local infections), Systemic infections such as pneumonia, bacteremia, sepsis, Infection by invasive device entry on(dialysis, intravascular catheters, Toxin associated diseases such as toxic shock syndrome and *Staphylococcal* Scalded Skin Syndrome etc. [1-10]

Probiotics are microorganisms that are found in the gastrointestinal tracts of both animals and humans (Vandenplas *et al*., 2015). Different probiotic bacteria recognized, include (*Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus acidophilus*) have been broadly usedas ingredients in dairy products. *Lactobacillus* is a type of friendly and probiotic bacteria that can be found in our digestive, urinary, and genital systems and does not cause illness. Also, it is including in some fermented foods, such as yogurt, and dietary supplements. Lactic acid bacteria (LAB) were found in abundance in a variety of naturally fermented foods. It is a natural remedy for raising young animals and humans, as well as for nutrition and sickness treatment (Arshad *et al*., 2018). *Lactobacilli* help to maintain the natural balance and stability of the microflora in chicken intestines. It decrease fat contents in the body and serum and improve the poultry performance. Also, it can be used as an alternative for antibiotics (Messaoudi *et al.*, 2013). *Lactobacillus* produced different secondary metabolites like organic acids (mainly lactic acid) and hydrogen peroxide (H_2O_2) that has antimicrobial activity. Bacteriocins are peptides created by ribosomes, specifically lactic acid bacteria with antimicrobial (bactericidal or bacteriostatic) activity as narrow spectrum or broad spectrum (Turovskiy *et al*., 2009; Simons *et al*., 2020). Among antimicrobial synthesized in the ribosomes, (lactacin B) from *L. acidophilus*, (plantaricin 423) from *L. plantarum*, (pediocin ST18) from *Pediococcus pentosaceus*, (nisin Q) from *Lactococcus lactis* and numerous from other bacteria. These bacteriocins were expected to inhibit the invasion of pathogens or competing strains, or regulate the composition of the microbiota and stimulus immune system in the host, LAB derived bacteriocins have been used as topical antibiotics, oral or disinfectants as stated by Messaoudi *et al.* (2013). [11-20]

MATERIALS AND METHODS

Lactic acid bacteria (*Lactobacillus* spp.)

Collection of samples and isolation of *Lactobacillus* SSP

Different samples were collected from different sources (food and clinical), 10 samples from the food sources (yogurt, liquid milk) and 15samples from clinical sources; (feces of healthy breastfeed infants, mouth of healthy infants and adults, and from vagina of healthy women were taken from private clinic). For the isolation from clinical samples, the swabs were immersed in 9 ml of MRS broth medium tubes and transferred to the laboratory in ice box. Each tube containing 9 ml of MRS broth was inoculated with 1 ml of liquid sample and mixed gently to get a uniform sample separately. All the inoculated tubes were incubated an aerobically at 37°C for 48 h. After incubation, large white colonies were selected, transferred and purified by streaking method on MRS agar plates and incubated. [21-33]

Refreshment of commercial brands of *Lactobacillus* probiotics

Probiotics were activated by dissolving them in MRS broth and incubating for 48 h at 37 °C and then mixing them lightly, streaked onMRS agar, and incubated for 24-48 h at 37 °C. This process was repeatedmore than once, until obtaining of single, pure colonies of bacteria. [34-40]

Identification of *Lactobacillus*

Morphological and microscopic examinations

Lactobacillus spp. isolation was carried out depending on the morphological characteristics of colony on (MRS) agar plates such as shape, color, odor and other characteristics (Goldman and Green, 2015). The microscopic examination was made for all bacterial isolates including; Gram staining that done to observe reaction to stain, cells shape and arrangement under compound microscope.

Biochemical tests

Catalase test

From each clinical isolates a single colony was smeared on a slide and drops of (3%) H_2O_2 were added. The appearance of bubbles indicated positive results.

Gelatinase test

Gelatin medium **(**It was prepared by dissolving 120 g of gelatin in 1000 ml TSB, and then dispensed into tubes (10 ml), autoclaved at 121°C for 15 minute. The medium was used to examine the ability of Lactobacillus to produce Gelatinase (Atlas *et al*., 1995)**.** Tubes were inoculated (except control tube) with 1% of fresh bacterial isolates (*Lactobacillus*) and incubated an aerobically at 37°C for 48 h, then tubes were kept in refrigerator (4 C) for 30 minute. Liquefaction of medium indicates positive result.

Screening for bacteriocins production by *Lactobacillus*

In order to screen *Lactobacillus spp*. isolates for their ability toproduce bacteriocins, primary and secondary screening techniques, which done with duplicate, were used as following:

Primary screening by:

Agar-plug diffusion method:

A volume of 0.1ml of 1.5×10^8 CFU/ml (McFarland tube No. 0.5) of fresh *Lactobacillus* broth culture was transferred and spread on MRS agar plates and incubated an aerobically at 37°C for 24-48 h. A 0.1ml of 10^8 cell/ml of the activated bacteria suspension was transferred and spread on MHA agar. Corn borer (6mm) was used to cut pieces of MRS agar which *Lactobacillus* grown on it, transferred to MHA agar and incubated aerobically at 37°C for 18-24 h. The formed inhibition zone around each plug was measured and recorded. None inoculated MRS agar was used as anegative control.

Agar-wells diffusion method

Lactobacilli were inoculated in MRS broth under anaerobic conditions at 37 C for 24-48 h. The cultures were centrifuged at 6000 rpm for 15 minute at 4°C. A 0.1ml of 10^8 cell/ml of the activated bacteria suspension was transferred and spread on MH agar. Wells cut into the pour plates with 6mm sterile cork borer were filled with 100 µl of the cell-free supernatant (CFS). The plates were kept at room temperature for 2 h and then incubated at 37 °C for 18-24 h. Finally, the inhibition zones formed around the wells were measured in mm, compared with that of control which contained MRS broth only.

Secondary screening by Bacteriocin ELISA Kit

Bacteriocin ELISA Kit

ELISA (Enzyme-linked immunosorbent assay) is an immunoassay technology that uses

antibodies to capture antigen and an enzyme-labeled antibody to quantify antigen concentration. Is a simple and rapid way was designed for the detection and the quantitative determination of bacteriocin in cell culture supernates. Components of bacteriocin ELISA kits used in this study are indicated in table-1:

Table-1: Components of Bacteriocin ELISA kit (Bioassay Technology Laboratory)

Components	Quantity
Standard Solution(ready to use)	0.5ml
Pre-coated ELISA Plate	96 well strips
HRP conjugate	6ml
(TMB) Substrate: (ready to use)	6ml
Stop Solution: (ready to use)	6ml
Wash Buffer Concentrate (25x)	20ml

Assay procedure:

1. The kits components were left at room temperature for 30 minutes.
2. About 100 µl of Standard working solution was added to the first 6 wells of the first column and 100µl of sample was add to other wells then covered and incubated for 60 m at room temperature with gentleshaking.
3. The solution was aspirated from each well and washed 3 times with 300µl of diluted washing solution.
4. A volume 100 µl of ready to use conjugate was added to each well and incubated for 30 minutes at room temperature with gentle shaking.
5. The solution was aspirated from each well and washed three times then 100µl of substrate solution was added to each well.
6. The plate was covered and incubated at room temperature for 15 minutes in the dark, then 50µl of stop solution was added to each well and mixed gently for 15-20 seconds.
7. The optical density of each well determined with a computerized microplate reader, and the results were read at 450 nm immediately.

Production of bacteriocin

Extraction of bacteriocin

The cell free supernatant was prepared according to method of (Aljeboury and Mahmoud, 2020), (100) ml of (MRS) broth medium were inoculated with (5) ml of LAB broth culture L7 , incubate under anaerobically condition at 37°C for 72h ,then centrifugation at (10000) rpm for 15 minutes at 4C. The precipitate was discarded while the supernatant was filtered Millipore filters in diameter (0.22µm). Cell free supernatant was collected and kept at 4°C until use and it was applied as a crude bacteriocin (Ogunbanwo *et al*. 2003). Protein concentration of the bacteriocin in the sample was determined by ELASA kit. [41-50]

Purification of Bacteriocins

Ammonium Sulphate

The cell free culture supernatant (crude bacteriocin) was saturated with 70% ammonium sulphate and stored at 4^0C to precipitate out the proteins. The pellet was collected after centrifugation at 10000 rpm at 4^0C for 30 minute. The pellet was dissolved in 0.1 M phosphate buffer (pH 7.0) and dialyzed.

Dialysis

The protein solution was removed by using dialysis bags was carried out with PBS buffer at 4°C

for 24 h with stirring and changing PBS for fourtimes to increase the efficiency of dialysis process then immersed the dialysis bags in sucrose powder to concentrate proteins and kept in refrigerator until the use.

Gel chromatography

Concentrated bacteriocin sample was further purified by gel filtrationusing Sephadex G-100. Five g of Sephadex G-100 was activated according to the standard procedure. Further swollen, deaerated gel was packed in column (1.5 x 70 cm) and column is washed with 0.1 M phosphate buffer(pH 7.0). Concentrated dialyzed bacteriocin sample was loaded on the packed column, then it was eluted with 0.1 M phosphate buffer (pH7.0) and samples fractions of 3 mL were collected at the rate of 0.25mL/min. Protein content of fractions were analyzed by measuring its absorbance at 280 nm and for bacteriocin plate assay were used. [51-58]

Biological Applications of Bacteriocin

Effect of Bacteriocin on bacterial growth

Agar-well diffusion method

Bacteriocin as an antibacterial agent was applied as follows: using Agar-well diffusion method according to Archana and Abraham, (2011)

- Muller-Hinton agar plates were inoculated with indicator isolates (*S. aureus*) by using a few colonies from overnight culture transfer to five mlof normal saline in order to prepare the bacterial suspension, which was adjusted to 0.5 McFarland turbidity equivalent to 1.5×10^8 CFU/ml.
- Spread bacterial suspension by sterile cotton swab on all Muller- Hinton agar surfaces, the swab was streaked across the medium surface.
- Using a sterile cork borer, five wells of about 6 mm diameter wereaseptically cut on the agar-plate.
- A volume of 100µl of each 100 %, 50%, 25%, 12% of Bacteriocin was added in to the each well by micropipette. A control well was made in thecenter with the D.W.
- The plates were incubated overnight at 37ºC and the diameter of any resulting zones of inhibition was measured in millimeters.

Microtiter plates method

1. In this method, 96-well microtiter plate was used (Sakagami and Kajimura 2002).
2. Each well was filled with 125 µl of Muller-Hinton broth
3. A micropipette was used to add 125 µl of bactericin stock to the first well.
4. A volume of 125 µl was transferred by micropipette from the first well to the second well and from the second well to the third well and so on to the last well, disregard125 µl from the last well.
5. By micropipette 5 µl of the bacterial suspension was transferred to all the wells, with two repetitions for each isolate.
6. A negative control (a culture medium and bacteriocin free of bacterial growth) was prepared.
7. The plate was closed and parafilm was used to prevent evaporation ofthe contents of the wells and incubated at 37°C for 24 h.
8. The results were recorded after incubation.

Reading the results:

- The results were read by examining the turbidity formed at the bottom of each well
- By a loop a portion of bacterial suspension from each well was transferred carefully and

evenly spread on BHI agar, and r then incubated at 37 °C for 24 h to observe the growth

- About 10 µl of Resazurin sodium salt dye prepared according to paragraph. were added to each well in the microtiter plate and incubated at 37°C for 2-4 h to notice the color change as evidence of the positive results (figure-1).

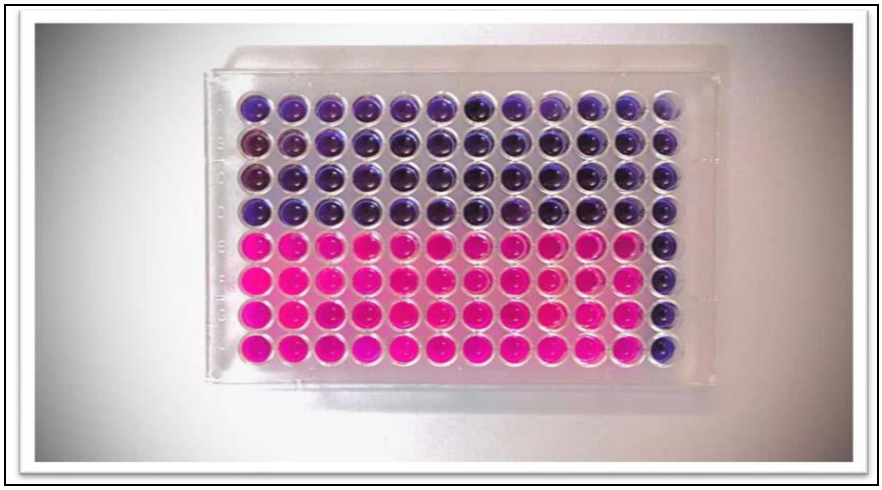

Figure-1: Determination of minimum inhibitory concentration by resazurin microtire assay method (pink colour indicates growth and blue means inhibition of growth).

Influences of bacteriocin on biofilm formation

The effect of bacteriocin as anti-biofilm was achieved by using micro titer plates with 96 U shaped-bottomed wells as identified by Harmsen *et al.* (2010), as explained below:

1. The first three Wells of plate contained 200 µl of B.H.I with glucose1%only considered as negative control
2. A volume 100 µl of broth(B.H.I. with1% glucose)and 100 µl of bacteriocin were added In the rest of the wells
3. Transferring 5 µl of the bacterial through a micropipette to all the wells, with three repetitions for each isolate.
4. The plate was closed and parafilm was used to prevent evaporation of the contents of the wells and incubated at 37°C for 24 h.
5. By washing the wells three times with PBS unattached bacterial cells were removed and then left to drying at room temperature for 15m.
6. 200 µl of Crystal violet section was added to the wells for 20 min. After removing the crystal violet solution wells were washed three times with PBS to remove unbounded dye and allowed to dry at room temperature.
7. The dye bound to the adherent biofilm was resolubilized with 200 µl ethanol 96%.
8. Optical density (OD) of the stained adherent bacteria was determined byELISA reader at a wavelength of 630 nm to detect the ability of bacterocin as anti-biofilm.

RESULTS

Isolation and identification of *Lactobacillus*

Morphological, microscopic and biochemical examinations

After cultured on MRS agar containing calcium carbonate as a result of dissolving by the acid produced by the bacteria, 12 of the 30 samples collected from various sources were able to grow in De Man-Rose-Sharpe (MRS) medium. MRS is a highly selective medium for detecting Lactic Acid Bacteria (LAB). These isolates were first detected by the creation of a transparent zone around their expanding bacteria colonies on MRS medium. These traits are attributable to *Lactobacillus* colonies, which are small white to creamy large colonies with regular edges, slight

mucoid, round form, convex, and have a strong odor. Lactic Acid Bacteria (LAB)cells stained positively with Gram stain and appeared as purple rods with round ends that occurred singly, in pairs, or in short chains and were non-spore producing. All of the bacterial isolates tested negative for catalase, oxidase, and Gelatinase.

Screening for bacteriocins production by *Lactobacillus spp*.

All *Lactobacillus* isolates were exposed to primary and secondary screening in order to determine the most efficient bacteriocin producing isolate to be applied for the next experiments.

Primary screening

The antibacterial activity of *Lactobacillus* against *S. aureus* isolates was determined using agar-plug diffusion and agar wells diffusion methods as a primary screening. The results showed that agar-plug diffusion appear had a little effects as antibacterial on pathogenic bacteria while agar well diffusion method appeared to be more efficient on the same bacteria.

In agar-plug method Only 6 isolates exhibit inhibition zones against 16 *S. aureus* isolates, with inhibition zones ranging from 4 to7 mm in diameter. While the other isolates of *Lactobacillus* did not showed any antimicrobial effects since non inhibition zones have been recorded. The results of the agar well diffusion showed that only 10 isolates of *Lactobacillus* (L1, L2, … and L10) express antibacterial activity against the 16 tested bacterial isolates and that isolate numbered L7 was the best one, thatCFS of LAB (L7) recorded the highest inhibitory activity with an average diameter of inhibition zone (8.0) mm.

The agar wells diffusion method was found to be more accurate than other method since it recorded an inhibition zones between (9 and 16) mm while in the agar-plug method, the diameters of inhibition zones were between (4 and 7) mm. According to Noordiana *et al*., (2013) *Lactobacillus ssp.* Antimicrobial activity is related to its ability to produce of inhibitory substances such as organic acids, bacteriocin, (CO) and (H_2O_2), as well as other substance such as bio-emulsifiers. Lactic acid bacteria have an inhibitory effect due to the lactic and acetic acids produced during fermentation, as well as a significant number of metabolites with antibacterial and antifungal activities. Free fatty acids, formic acid, ammonia, diacetyl, hydrogen peroxide, ethanol, acetoin, acetaldehyde, 2,3-butanediol, benzoate, bacteriocins, and bacteriolytic enzymes are examples of these chemicals (Cizeikiene *et al.,* 2013; Rocha and Malcata,2016; Bartkiene *et al.,*2020).

The qRT-PCR method has been used to examine the expression levels of the *OmpA* gene in *Acinetobacter baumannii* isolates under the effect *Lactobacillus casei* and *Lactobacillus plantarum*. The results showed that the *L.casei* and *L. plantarum* increased the transcript levels of the tested gene in *A. baumannii* isolates (Shareef *et al.,* 2021). Aljeboury *et al.,* (2019) studied role of probiotics (*Lactobacillus plantarum* and *Lactobacillus acidophilus*) in the treatment of *Helicobacter pylori* infection and study of effect of probiotic on reducing *H. pylori* stomach ulcer as supporting and safer.

Depending on the results obtained, the most efficient isolate for secondary screening. The results of this procedure were used to select the best isolates for secondary screening. It's worth to mention that our results were in accordance with previous published results of Aljeboury and Mahmoud in 2020, who reported the ability of cell-free supernatants of LAB to inhibit both gram positive and gram negative bacteria, including *E. coli, Klebsiella sp, Salmonella sp, and S. aureus.*

Another study by Bartkiene and his colleagues in 2020 recorded antibacterial activity for thirteen tested LAB strains against fifteen pathogenic strains including *(Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica, Acinetobacter baumanni, Staphylococcus aureus).*

Secondary screening

Measurement of Bacteriocin in LAB by ELISA Kit

ELISA Kit was used to measure the protein concentrations and confirm their presence in the selected isolates; then the most efficient isolate was chosen and purified by gel chromatography. it was confirmed that the crude extract contained the active compound of bacteriocins by ELISA. A clear color change was obtained on the ELISA kit, evidence of the compound's presence regardlessof its concentration.

The results showed that most isolates produced Bacteriocin but in different concentrations with the fact that the isolate no. L7 gave the highest concentration of bacteriocin and this result confirms the primary screening that showed the ability of LAB (L7) to inhibit MDR *S. aureus.*

Purification of Bacteriocins

Bacteriocin produced by *L. plantarum* (L7) that express a wide spectrum of antimicrobial activity was further subjected to a purification process. Two step purification processes was carried out; firstly partial purification was achieved by subjecting cell free supernatant to ammonium sulfate precipitation at 70%. Further purification was carried out by size exclusion chromatography using Sephadex G100. One saturation rates of ammonium sulphate (70%) were used for bacteriocins precipitation; the results showed that the protein concentration by *L. plantarum* L7 was (810 U/ml).

Proteins are soluble in aqueous media because they have hydrophilic amino acid side-chains which are supplied by the essential amino acids. A salt like ammonium sulfate interferes with these interactions between amino acid side-chains and water, through reducing the available water and will reduce the solubility of the protein. This enables protein interactions instead of protein-water interactions and the protein will come out of solution. Depending upon the hydrophobicity of the protein, different proteins would separate at different ammonium sulfate saturation levels. Higher hydrophobicity and higher ammonium sulfate concentration would be required to break the protein-water interactions to enable its precipitation (Burgess, 2009).

Gel filtration chromatography technique was the final step in the purification of bacteriocins produced by the local isolates *L. plantarum* (L7). After purification by ammonium sulfate step, fractions representing bacteriocins were collected, pooled and concentrated to be applied in Sephadex G 100previously equilibrated with 0.1 M phosphate buffer (pH 7.0)

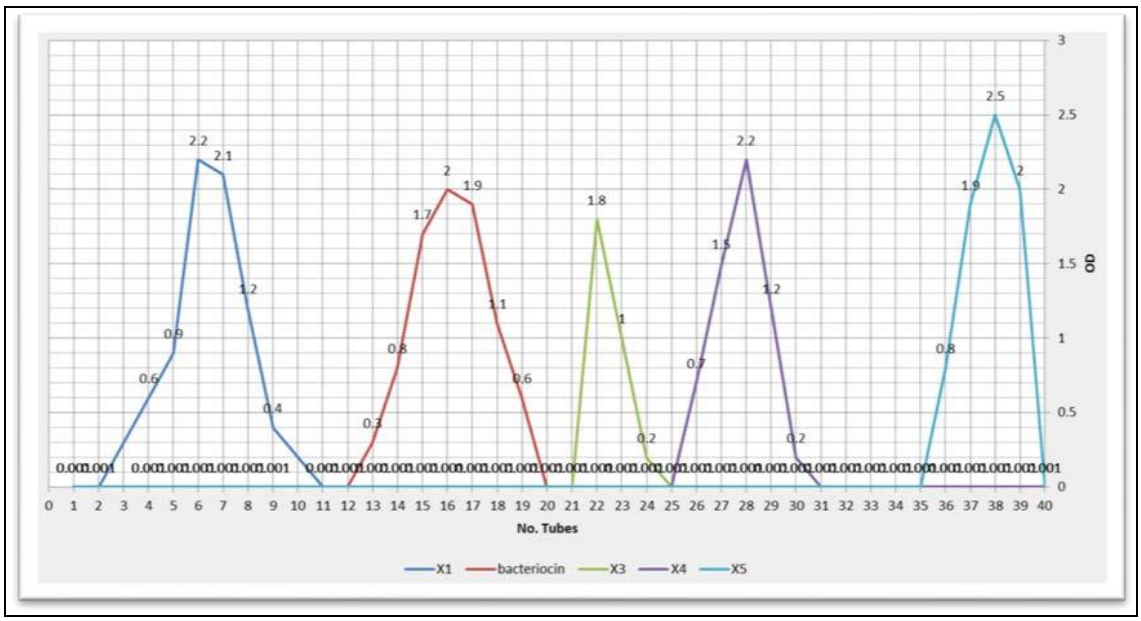

Figure-2: Gel filtration chromatography for purified Bacteriocin using Sephadex G-100 column.

Results illustrated in Figure (2) showed that there is only one absorption peak which represents bacteriocins after elution with phosphate buffer (pH 7.0). Protein concentration of bacteriocins were measured by ELISA (sandwich ELISA methods). In this figure, five peaks were drawn with chromatography column (X1, X2, X3, X4 and X5), and the highest peak wasX2 with highly concentration of bacteriocin 3732 U/ml as compared with other peaks (X1=0.79, X3=0.145, X4=0.145, X5=0.75 This is a results showed high concentration in fraction number between (12 - 18). The fractions were high specific and sensitive to antibody that coated in ELISA kit , therefore these fractions were selected to have pure bacteriocin for preparation used in next steps and other biological applications.

Antibacterial Activity of Bacteriocins on *Staphyloccousaureus*

Depending on the formed inhibition zone, the antibacterial activity of bacteriocin extracts from *Lactobacillus* at (100%, 50%, 25%, 12.5%) concentration against sixteen *S. aureus* isolates were tested. Results showed that bacteriocins had an effective antibacterial activitythat increased with increasing concentration with an inhibition zone ranged (21-23 mm) in diameter at (100%) concentration. And for (50%) concentration, the antibacterial activity against *S. aureus* isolates was recorded with an inhibition zone ranging from (9-11 mm) (table 2). While at (25%, 12.5%) concentration no effect was detected against the same tested bacteria, figure (3).

Table-2: The impact of different concentration of bacteriocins on *Staphylococcus aureus*.

Concentrations of Bacteriocins	Inhibition zone (mm)
100%	21-23
50%	9-11
25%	-
12.5%	-

Bacteriocins are known to inhibit various bacteria and their biofilm. They are ribosomal synthesized peptides that play as anti- microbial activity against bacteria with the fact that the producer strain itself is resistant to bacteriocins through the action of its resistance genes. These strain resistance is mediated by specialized ATP binding cassette efflux transporters or antagonistic bacteriocin receptors. Bacteriocin was stable at wide ranges of pH (2–12) and temperature(30–121°C), but it was sensitive to four proteolytic enzymes (Sharma *et al*., 2018). The organic acids especially (lactic acid) which produced by LAB, play important role in inhibiting *S. aureus* growth *Staphylococcus aureus* (MRSA) is an important resistant pathogenic bacteria that are difficult to treat with increasing death risk. MRSA strains are commonly resistant to β-lactam antibiotics and other drugs such as (aminoglycosides, macrolides and lincosamides).

In addition, the ability of bacteria for biofilm formation on surfaces of hospital and devices lead to high antibiotic resistance and transfer of genetic resistant. Here, we selected 16 clinically *S. aureus* isolate their resistance to six antibiotics and ability to form strong and moderate biofilm. The results indicated higher properties for bacteriocin, and confirm the results described by a number of researchers like Othman*et al.,* (2017) and Pato *et al.,* (2021) recorded the ability of bacteriocin to inhibit the growth of *S.aureus*.**.**

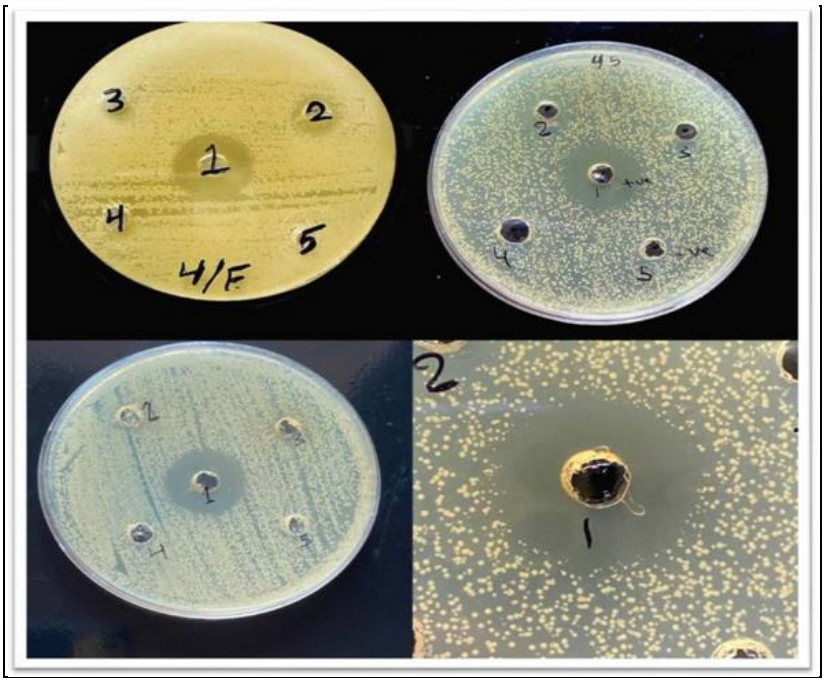

Figure (3): Antibacterial activity of bacteriocin produced by *Lactobacillus* ssp L7 against *Staphylococcus aureus*.

Inhibition of Biofilm formation by bacteriocin

The obtained results showed that bacteriocin at concentration of (100%) could inhibit all the *S. aureus* isolates (strong and moderate biofilm producers). Thus, bacteriocin has antibacterial activity against *S. aureus* strains, a result that previously recorded by Similar with De Giani in (2019) whoshowed a broad spectrum activity for plantaricin P1053 against *S. aureus* and *E.coli*. Lactobacillus, due to its diversity can yield diverse bacteriocins with different activity (Goyal *et al*., 2018). All bacteria in a biofilm matrix are highly resistant to antibiotics. Therefore, many efforts have been made by scientists to find new therapeutic strategies for inhibition of biofilm (Mathur *et al*., 2017). In recent study Mohapatra and Jeevaratnam (2019) showed the antibiofilm action of bacteriocinogenic (*L. plantarum*) against *S. aureus* and *P. aeruginosa.*

Various mechanisms are involved in the process to adhesion the biofilm on the surface, such as the presence of genes and production of extracellular polymeric substance. Therefore, the developing of new strategies to inhibit bacterial infections caused by biofilm depending on the four stages lifecycle of the biofilm include (inhibition of bacterial adhesion; weakening the biofilm maturation; disorder the advanced stage biofilms; and killing that relates with the eradication of biofilm). The activity of bacteriocins is usually bactericidal; therefore, they could be an alternative to modern antibiotics for treating biofilm related bacteria.

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