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The study of the efficacy of bacteriocin isolated from the genus *Salmonella* and its role in treating Basra water pollution

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Abstract

The study includes the production of bacteriocin from *salmonella spp* bacteria isolated from different food products, and the effect of crud bacterocin against gram positive and negative bacterial isolates from tap water for different areas of Basra city in Iraq. Salmonellacin a wide range of activity against closely related strains of anti-gram positive bacteria, with inhibition diameters ranging between 11-15 mm. The optimum conditions for production were at a neutral pH at a temperature of 37 for 72 hour.

Introduction:

Waterborne diseases are considered as major cause of mortality in the world, especially in less developed communities [1]. Data collected from international agencies indicates that at least 15% of the world population lack the access to uncontaminated water [2]. As a result, many diseases, including diarrhoea, cause the mortality of about 2.2 million persons, in which the majority are children below 5 years of age annually [3]. Thus, due to substantial importance of water contamination and the fact that water is uniquely exposed to contamination, measures of microbial control were strengthened and acceptable upper level for total coli contamination in drinking water is currently set to 100 CFU / ml [4].

A technique chromatograph that utilizes small particles by magnetic force to separate, in which the column can be used and cannot produce wastes secondary [5]. To increase the efficiency of the separation, it is preferred that the magnetic force is as large as possible. The magnetic force increases in accordance to the gradient of the magnetic field [6]. In general, the water activity has low and do not offer adequate growth curve bacterial [7]. Yet, *Salmonella* has already been isolated from various nuts like (peanuts, pecans, almonds, and pine nuts [8,9].

Salmonella Food sources of poisoning are mainly including milk, eggs, meat (poultry, beef), vegetables & fruit. To limit *Salmonella* species food borne illness, a worldwide monitoring and controlling methods are needed to be improved [10]. Bacteriocins structure protein or peptidic toxins produced by the different species bacteria to inhibit the growth of

similar or closely related bacterial strains. They are similar to the killing factors produced by yeast and Paramecium and are structurally, functionally and ecologically diverse. Thus, bacteriocins are currently tested to assess the possibility of their use as narrow-spectrum antibiotics [11].

Aim of his study was conducted to assess the prevalence, distribution and identification of *Salmonella* serotypes in different food samples, along with evaluating the effectiveness of the bacteriocin produced by this genus against water pollution causing bacteria.

Materials and methods

Culture media used in the study

The media used in this study, including nutrient agar, nutrient broth, brain heart infusion broth, blood Agar, Salmonella-Shigella agar (SSA), as well as Mueller-Hinton agar (MHA), were prepared according to instructions provided by the manufacturer. Once prepared, media were brought to a boil to dissolve all constituents completely. They were incubated at 37˚C to check sterilization efficiency for (18- 24) hrs.

Food samples collection

Forty different food samples in total were purchased from local markets in Baghdad in the period between October 2019 to January 2020 as shown in Table (1). Records of the labelling information and commercial names were taken when samples were collected. Samples were transported to the microbiology laboratory, using a cooler to prevent any excess bacterial growth and to proceed in the isolation of the pathogenic bacteria of interest on the same day.

Table (1): Distribution of study food samples.

Type of sample	No. of Sample
chocolate bars	6
Nuts	8
Cake	7
Arab Cheese	8
Kemar Arab	8
Raw milk	3 (Different places in Baghdad city)
Total	40

Antimicrobial susceptibility testing

The test for screening the isolates bacteria from water sample were regarded depended on CLSI criteria [12]. To assess the virulence of the bacteria isolated from tap water regarding their antibiotic resistance, antibiotic sensitivity test was performed for each isolate utilizing Kirby-Bauer (disc diffusion method) method. This assay was culture on MHA with the following discs antibiotic described shown Table (2). Following for 24 hrs. at 35ºC, results of the sensitivity test was read.

Table (2): Antibiotic Discs for Antibiotic Sensitivity test

Antibiotics (μg)	Company
Augmentin, (AU) 10	Bioanalyse (USA)
Vancomycin, (V) 5	Bioanalyse (USA)
Methecillin, (ME) 25	Bioanalyse (USA)
Gentimycin, (G) 10	Bioanalyse (USA)
Azithromycin, (AZM) 15	Bioanalyse (USA)

Optimization for growth

Temperature, PH, salinity, and media are considered to affect the extraction of bacteriocin [13]. Therefore, changes in the pH 7, salinity 0.5%, carbon substrates like source, glucose (1.0%), and peptone (0.5%) which affect growth, and monitored 6 hrs.

Preparation of crude bacteriocin from Salmonella spp

To screening bacteriocin from five strain *Salmonella typhi* isolated from Raw milk, 10 ml aliquots of Muller Hinton Broth (MHB) were prepared as previously described and used to culture primary inoculum. After incubation for 24 hrs. at 37C bacterial cells were removed by at 10,000rpm centrifugation. We separated supernatant and it was adjusted pH to 6.5-7.0 using 1 N NaOH and then filtered using 0.22 µm membranes and stored at 4 °C till use [14], and Protein estimation according to [15].

Detection of bacteriocin antibacterial activity

Firstly, experimental strains *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* isolation form tap water, and maintained in nutrient agar slants for 24 hours prior to the experiment cultures were thawed to room temperature. The inhibitory activity of crud bacterocins produced from five strains against target organisms were tested on Muller Hinton Agar medium by agar diffusion assay [16]. Incubate the plates at 37^0C for 24 hours. Inhibition zones around the wells were measured and recorded and the antibacterial activity was assessed according to previous study [17].

Determination of the Optimal Conditions for Bacteriocin Production.

In order to determine many conditions that support the maximal production of bacteriocin [18].

Water sample collection

The drinking water used in this study was collected from the river supplied to different suburbs of Al-Basra city including, Al Mueaqil, Tanumia, Al Matiha , and Al Khalilayuh, which is considered as surface water. The source of the drinking water of these suburbs is (Ashar Rive) , which is known to be contaminated with industrial and urban waste water. Thus, it is possible that the drinking water is contaminated as well, which would elevate the exposure to different health risks by the individuals. Since one of the sources of contamination is the microbial contamination, drinking water samples were collected from these four

geographically different suburbs of Al-Basra province, with 5 replicates for each suburb in1000 ml glass bottles.

Bacterial isolation and identification

Each sample of food put 10g or 10 ml according to type of sample then diluted in 90ml sterile enrichment peptone water and incubated at 37°C for (18- 24) hrs. Then loopful of incubated culture was cultured on were cultured first on salmonella shegiella agar and MacConkey agar.The identification of bacteria like, *Salmonella spp., and Staphylococcus aureus, Enterobacter spp, E. coli, Pseudomonas aeruginosa* and *P.mirabilis* were isolation from tap water.

Estimation of coliform in water samples

Coliform bacteria were determined the concentrations of by using 2 × 10 ml filtering for sample filters and pore size of 0.45 µm, then cultured on FC agar and m-Endo agar and Chromogenic Escherichia coli/Coliform medium (CECM) for fecal and total coliforms detection and enumeration respectively.

Results and Discussion

Bacteria Isolation:

Aseptically, water samples from the Basra city were collected on glass bottles is sterile from different points by dipping the bottles directly into the surface of the water. Into the sterile bottles purified water samples were poured from the tap and transported to the lab on ice for further analysis. A selective isolation was conducted on aliquots of these samples were used to detect fecal coliforms, total coliforms, Aeromonas, bacteria, utilizing standard microbiological procedures. Water samples collected from different suburbs in Basra as shown in Table (1) and Figure (1).

Table (1): The prevalence of bacterial species isolated and identified in Basra city water.

Region	Bacteria					
Almueaqil	*E.coli*	*Enterobacter aerogenes*	*Proteus mirabilis*	*klebsiella sp*	*Salmonella sp*	*S. aureus*
Tanumia	+	+	+	-	+	+
Almatiha	-	+	_	+	-	+
Alkhalilayuh	-	+	+	+	+	-
Tank water hulu	+	-	-	-	-	+

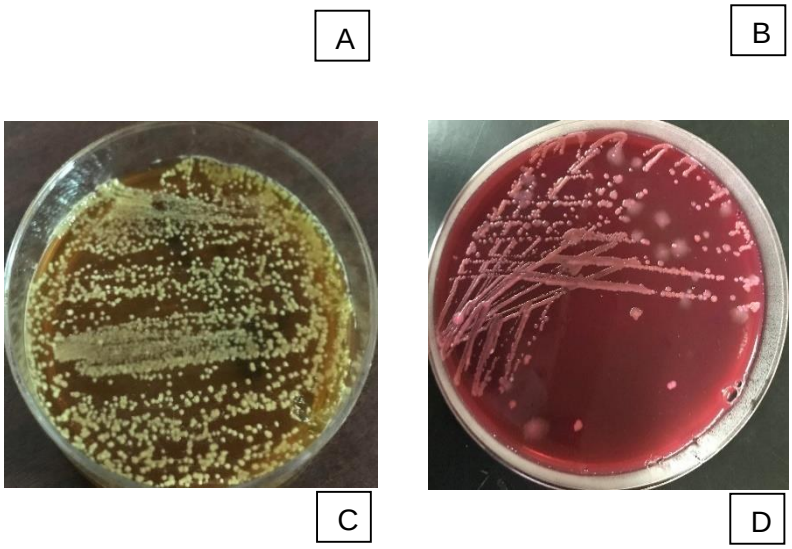
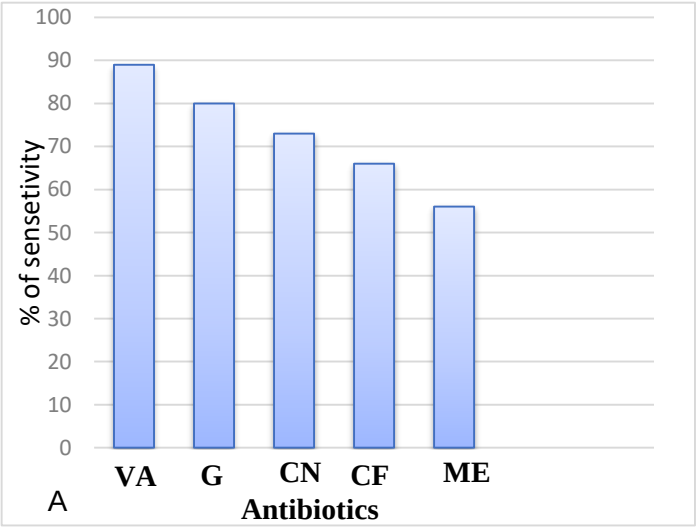


Figure (1): Images represent different bacterial species isolated from water and cultured on different media, where (A) *E. Coli* was cultured on MacConkey agar, (B) *Salmonella* on S.S agar, (C) *S. aureus* on MSA agar, whereas (D) *Enterobacter aerogene* was cultured on MacConkey agar medium.

Antibiotics sensitivity test (AST)

Figure (2) illustrates isolated bacteria the antibiotic resistance patterns of from water. The results showed a high resistance to antibiotic in gram negative bacteria like Gentamycin (89.21%), Amikacin (82.35%) and Ceftazidime (80.39%). In contrast gram positive bacteria exhibited a high resistance to Methicillin (74.50%) and Gentamycin (80.1%) and were sensitive to Vancomycin and Augmentin at (89.21%).



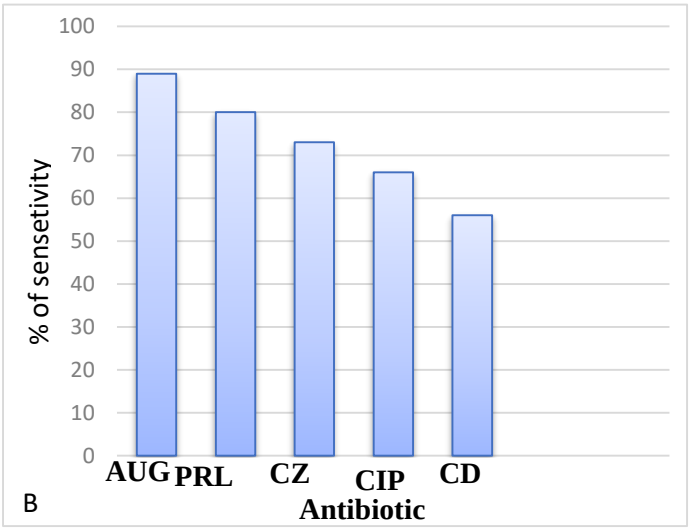


Figure (2): Antibiotic susceptibility test of A) Gram positive and B) Gram Negative bacteria isolated from water

Screening bacteriocin

Bacteriocins from five species *Salmonella spp* screening the best strain produce bacteriocin (Salmonellacin) from Raw milk are proteinaceous antibacterial compounds and exhibit bactericidal activity against closely related and different strains. Many bacteriocins are active against pathogens bacteria isolation from Al-Basra water. widest inhibition zone which reached between(11- 15)mm on the related type gram negative bacteria while the dimeter inhabition zone anti gram positive isolates reached (9-10)mm . was chosen as a good crude bacteriocin producer among all five stain. See table (2)

Table (2): Antimicrobial Activity of Salmonellacin against different Gve- and Gve+ bacteria isolated from Water.

Bacterial Strains	Zone of inhibition (mm)
Staphylococcus aureus	8
Pseudomonas aeruginosa	11
Salmonella typhi	15
E. coli	12
P. mirabilis	14
Enterobacter aerogenes	10

Optimization of bacteriocin :

The bast Salmonellacin produced among five stains optimization against indicator strain by detection dimeter of zone inhibitor. see fig (3)

- **Determination of pH for the optimum**
  The different pH effect on production of bacteriocin rang of pH as (4, 5 , 6, 7,8) dimeter reached 16 mm at pH 7 .
- -**Determination of inoculum size**
  Producer by using different inoculum (3 × 10^8 CFU/ml), (6 × 10^8 CFU/ml), and (9 × 10^8 CFU/ml) standard for 24 hr. at 37º C.the optimum inhibition against test strain reached 15mm at (1.2 X 10^8)
- **Determination of the optimum incubation period** the incubation period was testing at incubation time different between (24, 48 and 72) hr. the dimeter reached 16 mm after 72 hrs.
- **Determination of the optimum temperature** Study the effect of incubation temperature (25, 30, 37 and40) ºC the best inhibitor zone at 37c reached 17mm

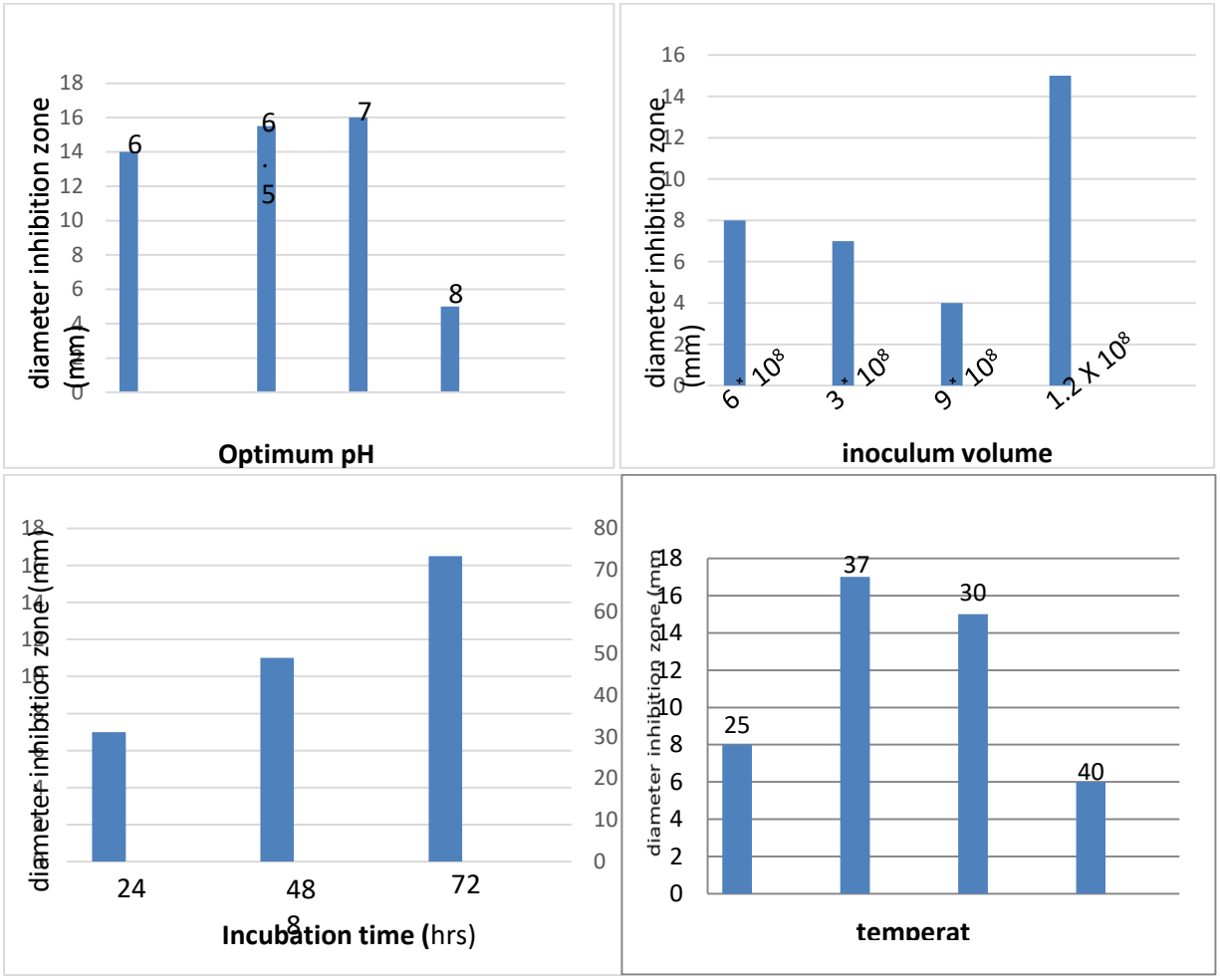


Figure (3): Determination optimum conditions for bacteriocin production

Salmonella spp one important strain isolated included (water and food possining) and major causes problem worldwide [19].

The results agree with [20] who found the well diffusion assay as the best method to screening bacterocin against test microorganism

According to [21] has shown bacterocin salmonellacin can be used different application show bio preservative and water treatment by inhibiting the growth of bacteria. Avarious physicochemical factors can influence bacteriocin production in environment such pH.

Using the well agar assay method in our study for evaluating the inhibitory activity of bacteriocin extracted from *Salmonella typhi* had shown a significance against harmful disease-causing bacteria such as *Enterobacter aerogenes* and *Salmonella typhi* and to a less extent action against *Proteus mirabilis* and *S. aureus*. [22]. This result disagree with [23] who produced on bacteriocin from *E. faecium* the optimum condation at 25˚C and activity bacterocin that the highest from *E. faecalis* after 24hr. The result agrees with [24] bacterocin from streptococcus spp more sensitive to acidity compared alkalinity and natural.

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دراسة كفاءة البكتريوسين المعزول من جنس السالمونيلا و دوره في معالجة تلوث ماء البصرة

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البكتريوسين، السالمونيلا، البصرة

معلومات المؤلف

الايميل:

الموبايل:

الخلاصة:

تضمنت الدراسة انتاج بكتروسين من بكتريا سالمونيلا المعزولة من منتجات غذائية مختلفة واظهرت الغربلة للإنتاج ان أفضل عزله كانت من عينات الحليب، ودراسة تأثير البكتروسين الخام المنتج ضد العزلات البكترية الموجبة والسالبة المعزولة من ماء الحنفية لمناطق مختلفة من مدينة البصرة في العراق. واظهر السالمونيلاسين فعالية واسعه المدى ضد السلالات المقاربة من البكتريا الموجبة باقطار تثبيط تراوحت ما بين 11-15 ملم. وكانت الظروف المثلى للإنتاج عند رقم هيدروجيني متعادل بدرجة حراره 37 لمدة 72 ساعة.