# Influence of chemical and physical conditions on the production of bacteriocin by Aeromonas hydrophila

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## Abstract:

Aeromonas hydrophila have been isolated as a cause of a cute gastroenteritis in 23 (5.6%) of 410 patients. Other bacterial enteropathogens have been isolated from 387 patients with diarrhea, were 19 different strains. A. hydrophila occurred more commonly in children with acute diarrhea, the results showed that 18(78.26%) isolates of A. hydrophila found in children under 10 years old ,distributed to 10(43.47%) in male and 8(34.78%) in female ,and in adults with diarrhea 5 (21.73%). In the other hand, we noticed frequency of isolation was higher in male 14(60.86%) when compared with 9(39.14%) in female. Six strains of A. hydrophila have been observed to have bacteriocin activity against 12 of 23 different A. hydrophila ,as well as Staphylococcus aureas, Pseudomonas aeruginosa, Escherichia coli, Enterobacter cloacea and Shigella dysenteria. The results showed Bacteriocin-like substances ($BLS_{11}$) had isoinhibitory activity on 10 same A. hydrophila species and heteroinhibitory activity effects on all pathogenic bacterial strains used, while $BLS_5$ showed isoinhibitory activity on 2 same A. hydrophila species and heteroinhibitory activity by effecting on gram negative only, and $BLS_3$& $BLS_{12}$ showed activity on E. coli isolates only, and none of $BLS_1$& $BLS_{10}$(isoinhibitory activity on 1 A.hydrophila respectively) had effect on all pathogenic bacteria. Among the standard laboratory media used Brain Heart Infusion broth (BHI) showed the maximum production and poor yields resulted from growth in Peptone Glyserol (PG) and Nutrient broth. We selected $BLS_{11}$ to their wide range effect on same species and enteric pathogenic strains, to study the Influence of chemical and physical conditions on the production of BLS by A.hydrophila. The $BLS_{11}$ preparations from A.hydrophila11 strains of A. hydrophila were tolerant to all three treatments of surfactant. In the other hand, effect of organic acid on BLS production $BLS_{11}$ has been studied and showed no remarkable difference in zone of inhibition when used acetone as affecter element, while both of isopropanol and ethanol have narrow inhibition zone range when compared with control strain. These results indicated that most A. hydrophila might be harboring plasmid mediated bacteriocin like substance, and there are no relation between BLS production and number of plasmid bands present in bacteria.


**Key words: Aeromonas hydrophila, Bacteriocin, Isoinhibitory activity, Heteroinhihitory acticity**

## Introduction:

Aeromonas hydrophila, a gram-negative, nonsporing, oxidase-positive, facultative rods that produce β-hemolysis on blood agar and ferment a variety of carbohydrates with acid and gas production [1]. A.hydrophila is the most common human pathogenic species of the Aeromonas genus (68%), followed by A. sobia (17%) and A. caviae (10%), according to a previous epidemiological report [2].Avariety of virulent factors have

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been described among the Aeromonas, such as enterotoxins (heat-labile and heatstable), cytotoxins, hemolysins and hemagglutinins [3]. The cytotoxin has been known to have a DNA homology and immunologic cross-reactivity with cholera toxin, has long been known as an important pathogen of freshwater fishes. It is also recognized as a pathogen of warm-blooded animals, including human [4] .In humans,some Aeromonas species have been associated with intestinal and extraintestinal infections and enterotoxins, cytotoxins as well as invasive mechanisms have been incriminated in the development of illness in the host. These virulence determinants were involved sequentially in enabling the bacteria to colonize, gain, entry, establish, replicate, and cause damage in host tissues and to evade the host defense system and spread, eventually killing the host [5]. Phenotypic characteristics of Aeromonas spp. have been used to differentiate between environmental strains and those strains causing gastroenteritis; including the lysine decarboxylase, Voges-Proskauer and autoagglutination positivity tests, congo red and crystal violet uptake and the production of a cell-free hemolysin and cytotoxin[6]. Bacteriocin-like substances (BLS) were protein compounds produced by some bacteria showing antagonic activity against their own species (isoinhibitory activity - IA) or other non-related species (heteroinhibitory activity- HA), these substances have been widespread utilized in epidemiological studies as specific marker properties of bacteria, in the regulation of population dynamics in bacterial ecosystems and clinical treatment .As BLS has not been currently described in A.hydrophila in Iraq. The purpose of this study was to investigate their production in strains isolated from clinical sources. Furthermore, we sought to study the effects that salinity and medium pH have as well as effect of surfactant and organic solvent on bacteriocin activity were determined and determine their antimicrobial activities against some common human enteric pathogens .

## Materials and Methods:

**Fecal Specimens**:
All diarrheic stool specimens submitted by physicians to teaching Laboratories, a clinical reference laboratory, for routine microbiologic analysis have been screened for Aeromonas during periods, March–November, 2008 in Baghdad hospitals (Al-kandi and welfare teaching hospital). Specimens have been plated for Aeromonas within 2–3 days after submission. Stool in Cary-Blair transport media has been directly streaked to Deoxycholate-Citrate agar (DC), MacConkey agar (MC), Sheep Blood agar (SBA) containing 10 μg/mL ampicillin [7] and incubated at 35°C. Presumptive Aeromonas isolates were screened for standard phenotypic traits (β-hemolysis, oxidase positive, indole positive) and species identity has been determined by using the API-20E identification system (bioMérieux, France). To discriminate A.hydrophila Kligler test (Appearance of alkaline surface and acid butt after 24 h. at 37$^{o}$C demonstrated the presence of A. hydrophila) wase carried out [8].

**Detection of antibacterial activity:** Antimicrobial activity has been confirmed by using the agar spot test method. Seven mL of sterile BHI soft agar has been cooled to 47°C and mixed with 10 /lL of a cell suspension of bioassay strains (over night cultures). The soft agar has been poured then over the agar plates and cooled at room temperature for 30 min. After the

plates were solidified make 5 lL of culture free supernatant of test organism. The plates have been incubated at 37°C for 18-24 h. and examined for the presence of clear zone of inhibition of 2mm or more around the spot.

**Inhibitory activity:**

The antimicrobial activity of bacteriocin has been tested against the test organisms following the method described in[9]. A. hydrophila inoculated into BHI broth and incubated at 37°C, without aeration until mid logarithmic phase of growth. Aliquot of 10 μ l cell-free culture supernatant has been spotted on the surface of agar plate seeded with actively growing cells of the test organism. Plates have been incubated at the optimal growth temperature of the test organism.

**Sensitive/indicator cultures:**

The Gram negative sensitive cultures have been selected among the staphylococci from the clinical specimens obtained for bacteriocin screening. S.aureus has been used most of the time as sensitive culture, Gram negative bacteria used as sensitive culture were obtained from various sources. Some were obtained from clinical sources, Department of Microbiology, University of Al-Mustansiryah. List of Gram positive and Gram negative bacteria (used as sensitive cultures) is listed below.
S.aureus, P.aeroginosa, E.coli , ,E.cloacea ,S. dysenteria

**Growth conditions:**

Almost all the bacteria (whether Gram positive or Gram negative; producers or indicators) were grown at 37°C for 24h. in their respective culture media.

**Preliminary screening of the isolates for bactericin or bacteriocin-like inhibitory substances:**

The inhibitory activity of the isolates was determined by Agar well diffusion method**:** Pre-poured BHI agar plates have been overlaid with 3.0mL BHI soft agar containing 0.lmL ($2x10^8$cfu/mL) of the sensitive culture. Wells (5mm in diameter) have been cut into these agar plates and l00μL of the culture supernatants was placed into each well, and kept at 4°C for 10-12h. to allow the bacteriocin to diffuse into the agar. The plates were then incubated at 37°C for 24h and zones of inhibition were measured in mm diameter.

**Structural media for bacteriocin screening:**

MRS medium in [10] modified, containing 10 g/l glucose, , 5 g of yeast extract, 2 g of K2HPO4, 2 g of diammonium hydrogen citrate, 0.2 g of MgSO4·7H2O, 0.05 g of MnSO4·4H2O, 10 g of Nacl and 1 g of Tween 80 per liter (pH 6.8).

**Physical Characterization Effect of pH:**

Bacteriocin preparations were adjusted to different pH levels between 3.0 to 9.0 with 10mM NaOH or l0mM HCl,. Samples were maintained for lh. at 37°C. All the samples were then re-adjusted to neutral pH (pH 7.0) and assayed for activity by agar well diffusion assay [11].

**Effect of NaCl or\ the production of bacteriocins:**

A set of fresh Tryptone soya broth (TSB: tryptone, 1.5%; soya peptone, 0.5%) containing NaCl at a final concentrations of 0.5, 1.0 and 2.5 % has been inoculated with a fixed volume of inoculum of A.hydrophilia culture. Broth without NaCl solution

has been taken as control. The optical density, viable bacterial count and bacteriocin activity was determined after 12h. [11 and 12].

**Effect of organic solvents:**

Equal volume of TSB containing A.hydrophila culture were mixed with different concentrations of organic solvents (listed in table 5) including: methanol, ethanol, isopropanol,, acetone and chloroform in a final concentration of 1.0% pre-cooled at (4°C). All the organic solvents have been obtained from Sigma except chloroform which was obtained from BDH. Samples were stirred and incubated at 37°C, except acetone which incubated at 4°C, for 30min and evaporated in a rotary evaporator. Dried samples were dissolved in 50mM sodium phosphate buffer, pH 7.0 and assayed for antimicrobial activity [11 and 12].

**Effect of surfactants:**

The bacteriocin preparations have been treated with different detergents: Triton X-100 (Fluka, Switzerland), sodium dodecyl sulfate (SDS, Merck), tween 80 (BDH, London) at a final concentration of 1.0%. Controls consisted of either bacteriocin preparation or detergent in 50mM sodium phosphate buffer, pH 7.0, all samples and controls have been incubated at 37°C for 6h. and titer for bacteriocin activity were determined [13].

**Effect of various media on the production of bacteriocin or bacteriocin-like inhibitory substances** [14]:

The antagonistic activity of the isolates has been determined by stabbing in pre-poured brain heart infusion agar (Oxoid), nutrient agar (Oxoid), and incubated at 37°C for 18-24h. The plates were exposed to chloroform (to kill the producer cells), and 3.0mL BHI soft agar containing 0.lmL of ($2x10^8$cfu/mL) sensitive culture was poured over the plates and incubated at 37°C. After overnight incubation, zones of inhibition around producer colonies were measured and documented.

**Plasmid DNA Isolation:**

The alkaline lyses method [15] has been used for plasmid DNA isolation.

**Agarose gel electrophoresis**:

Agarose gel electrophoresis was performed in Tris-acetate buffer, pH 8.0. Gels contained 0.7% agarose, and electrophoresis was performed at 75 V for 6 h. [16]

## Results and Discussion:

Routine faces samples from a cute gastroenteritis cases have been processed on the following enteric differential media: deoxycholate-citrate agar (DC), MacConkey agar (MC). The samples have been also cultured on Blood agar with ampicillin (BA) ampicillin-resistant haemolytic colonies on BA were tested for oxidase activity. All media were incubated aerobically at 37 °C for 18–24 h. colonies that were typical for Aeromonas and that grew on one of the agars mentioned above were cultured on nonselective medium (such as blood agar) and examined for oxidase.

A.hydrophila have been isolated as a cause of acute gastroenteritis in 23 (5.6%) of 410 patients. identified and their microscopic-morphological, cultural and biochemical characteristics were determined. Our study demonstrated A.hydrophila is case gasteroentirites in Baghdad hospitals. This result is agree with Unambiguous convincing evidence suggests that some Aeromonas do cause gastroenteritis [17]. Andlová [18]

noticed that Aeromonas isolation from human faeces samples is difficult and its success depends on the culture method performed. A valid judgement as to how many Aeromonas are involved in diarrhoeal disease is only possible when an appropriate selective medium is used. In our study, watery stools, fever, and abdominal cramps were the most common symptoms, which is consistent with other [19].

Other bacterial enteropathogens isolated from 387 patients with diarrhea were 19 different strains. No bacterial enteropathogens were isolated in the control group of patients.

**Table 1: distribution of A. hydrophila according to gender and age group**

| Age group (Year) | No. 0f diarrhea patients | | No. A. hydrophila isolation | Gender | |
|---|---|---|---|---|---|
| | | | | Male | Female |
| 1 month-10 year | Male | 168 | 18 | 10 | 8 |
| | Female | 121 | | | |
| 10 -20 | Male | 20 | - | - | - |
| | Female | 10 | | | |
| 20 -30 | Male | 14 | - | - | - |
| | Female | 9 | | | |
| 30 -40 | Male | 15 | 2 | 2 | - |
| | Female | 12 | | | |
| 40 -50 | Male | 10 | 2 | 2 | - |
| | Female | 8 | | | |
| 50 -60 | Male | 9 | 1 | - | 1 |
| | Female | 9 | | | |
| 60 -70 | Male | 3 | - | - | - |
| | Female | 2 | | | |
| **Total** | | 410 | 23 | 14 | 9 |

Gasteroenteritis caused by A. hydrophila occurred more commonly in children with acute diarrhea, the results showed that 18(78.26%) isolates of A. hydrophila found in children under 10 years old ,distributed to 10(43.47%) in male and 8(34.78%) in female ,and in adults with diarrhea 5 (21.73%) A. hydrophila have been isolated, considered in high age group, 2(8.69%) in 30- 40 and 40 -50 ages group respectively .However, the frequency of isolation of Aeromonas spp. in adults was less (5) than in children (18). In another hand we noticed frequency of isolation was higher in male 14(60.86%) when compared with 9(39.14%) in female. (Table 1) our results agree with [20] which confirmed the presence of pathogenic A. hydrophila in children with gastroenteritis in the study area. As shown previously results, A. hydrophila have been detected in significantly higher numbers in children with diarrhea than in adult and controls. However, others have found no significant difference in the frequency of isolation of Aeromonas spp. from individuals with and without diarrhea [21].

The assays for the production of BLS were performed according to used A. hydrophila strains as BLS producers and BLS indicators. The culture supernatants obtained from 23 A. hydrophila isolates were tested for antibacterial activity against the same group of l A. hydrophila. Among them, six strains of A. hydrophila were observed to have bacteriocin activity against 12 of 23 different A. hydrophila, as well as S. aureas, P.aeruginosa, E. coli, E. cloacea and S. dysenteria. Our results showed $BLS_{11}$ had isoinhibitory activity on 10 same A. hydrophila species and heteroinhibitory activity effects on all pathogenic bacterial strains used, while $BLS_5$ showed isoinhibitory activity on 2 same A. hydrophila species and heteroinhibitory activity by effecting on gram negative only, and $BLS_3$& $BLS_{12}$ showed activity on E. coli isolates only, and non of $BLS_1$& $BLS_{10}$ (isoinhibitory activity on 1 A. hydrophila respectivly) had effect on all pathogenic bacteria (Table 2). Our result established these bacteriocins had inhibitory effects on closely related A. hydrophila bacteria and enteric pathogens. That agree with [22] they showed (BLS) production by A. hydrophila are protein compounds produced by some bacteria showing antagonic activity against their own

species (IA) or other non-related species (HA).

**Table 2: Effects of six bacteriocins on the growth of some bacteria on agar plates (BHIagar)**

| Indicator strains | $BLS_1$ | $BLS_3$ | $BLS_5$ | $BLS_{10}$ | $BLS_{11}$ | $BLS_{12}$ |
|---|---|---|---|---|---|---|
| A.hydrophila | 1*/22** | 3/22 | 2/22 | 1/22 | 10/22 | 3/22 |
| S. aureas | 0/1 | 0/1 | 0/1 | 0/1 | 1/1 | 0/1 |
| P.aeruginosa | 0/1 | 0/1 | 1/1 | 0/1 | 1/1 | 0/1 |
| E. coli | 0/1 | 1/1 | 1/1 | 0/1 | 1/1 | 1/1 |
| E. cloacea | 0/1 | 0/1 | 1/1 | 0/1 | 1/1 | 0/1 |
| S. dysenteria | 0/1 | 0/1 | 1/1 | 0/1 | 1/1 | 0/1 |

* The number of sensitive strains
** The number of strains tested

Our study focus in effect of different liquid media to induce BLS, we were tested two liquid media used extensively to induce producing bacteriocin in different bacteria, Among the standard laboratory media used (BHI broth) showed the maximum production and poor yields resulted from growth in (PG) and nutrient broth. The influence of growth media constituents on BLS production by A.hydrophila was studied. This study Similar with other [23] the used (TSB) which showed the maximum production and poor yields resulted from growth in peptone water and nutrient broth. While the highest levels of bacteriocin production (aureocins) by strains of Staphylococcus aureus occurred in BHI medium.[24]

Our result reach to use BHI broth as inducible BLS in A.hydrophila rather than PG, because their ability to induce BLS, While PG was effect to induce BLS but in low effect in some bacteria (Table 3).Whereas bacteriocin inhibitors were not detectable in bacteriocin-inactive liquid cultures of A.hydrophila, the possibility that BHI broth represses the synthesis of such inhibitors cannot be ruled out. It should also be mentioned that not all strains of A. hydrophila produce BLS in BHI broth (Table 3). For example, other strains (17 strains) were grown in BHI broth and did not produce any detectable BLS. This failure was not due to the inability of these strains to synthesize any BLS but we believe if suitable agar media satisfactory may be producing BLS, and that is confirm when used designed structural media (MRS medium) many strain produce BLS (A.hydrophila 9, A.hydrophila 21, A.hydrophila 14) but in very low inhibition zone (9-10mm). This inhibition zone observation may be from media containing 40 or 80 g l -1of NaCl resulted in a significant increase in specific production rates of bacteriocin-like activity [25]. Presence of complex carbohydrates increase ability to produce bacteriocin [26].The study select $BLS_{11}$ to their wide range effect on same species and enteric pathogenic stains, to study the Influence of chemical and physical conditions on the production of BLS by A.hydrophila.

**Table 3: Effect of different broth media to induce BLS production from A. hydrophila**

| Indicator strains | Broth media | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BHI broth | | | | | | PG | | | | | |
| | $BLS_1$ | $BLS_3$ | $BLS_5$ | $BLS_{10}$ | $BLS_{11}$ | $BLS_{12}$ | $BLS_1$ | $BLS_3$ | $BLS_5$ | $BLS_{10}$ | $BLS_{11}$ | $BLS_{12}$ |
| **A.hydrophila 1** | R | R | R | R | 12* | R | R | R | R | R | 10 | R |
| **A.hydrophila 2** | R | 12 | R | 11 | 18 | R | R | 12 | R | 11 | 10 | R |
| **A.hydrophila 4** | R | R | R | R | 21 | 12 | R | R | R | R | 11 | 10 |
| **A.hydrophila 6** | R | 15 | R | R | 19 | R | R | 13 | R | R | 17 | R |
| **A.hydrophila 7** | R | R | R | R | 13 | 13 | R | R | R | R | 13 | 10 |
| **A.hydrophila 8** | R | R | 15 | R | 20 | R | R | R | 15 | R | 19 | R |
| **A.hydrophila 10** | R | 10 | R | R | 23 | 12 | R | 10 | R | R | 15 | 11 |
| **A.hydrophila 18** | 15 | R | R | R | 12 | R | 15 | R | R | R | 12 | R |
| **A.hydrophila 19** | R | R | 12 | R | 17 | R | R | R | 12 | R | 16 | R |
| **A.hydrophila 22** | R | R | R | R | 12 | R | R | R | R | R | 10 | R |
| **S. aureas** | R | R | R | R | 17 | R | R | R | R | R | 20 | R |
| **P.aeruginosa** | R | R | 13 | R | 20 | R | R | R | 18 | R | 11 | R |
| **E. coli** | R | 16 | 15 | R | 15 | 20 | R | 12 | 15 | R | 12 | 16 |
| **E. cloacae** | R | R | 21 | R | 17 | R | R | R | 18 | R | 11 | R |
| **S. dysenteria** | R | R | 22 | R | 17 | R | R | R | 19 | R | 17 | R |

*Inhibition zone (mm) by using agar plate, R: No inhibition zone

Tables 4 show the results of antibacterial activity of $BLS_{11}$ at the pH values of 5.0; 5.5 and 6.0 as well as salt concentration 0.5, 1.0, 2.5. At the same time, growth intensity of A. hydrophila is shown in control mediums at the same pH values and salt concentration. Regarding pH, the maximum inhibitory activity has been observed at pH 3.0 followed pH 5.0 and minimum was observed at pH 7.0, while loose inhibitory activity at pH 9.0. The loss of activity at higher pH could be due to degradation of the molecule. regarding various salinity (NaCl %) tested 0.5% NaCl was found to be suitable rather than 2.5% NaCl . On the basis of these results, our study pointed that A. hydrophila BLS gave better effect in low pH and 0.5% salt concentration, so that it must be purifier in low pH (Acidic) to keep activity. Bacteriocin production was strongly dependent on pH, nutrient source and incubation temperature as claimed by [9].Various physicochemical factors seemed to affect bacteriocin production as well as its activity.

BLS production was strongly dependent on pH, nutrient source and incubation temperature as claimed in [27]. Various physicochemical factors seemed to affect bacteriocin production as well as its activity. Maximum activity was noted at pH 3 and 0.5% NaCI. From the results proved that it can be used in acidic pH, as the optimum pH for activity was found to be pH 3.0. It might be secondary metabolites. BHI broth seemed to be more suitable medium compared to PG broth for the bacteriocin production. pH results consistent with those reported in [28], where the bacteriocin characterized showed an antimicrobial activity at the acidic pH more than the basic pH.

**Table 4: Effect of different pH, Salt concentration on $BLS_{11}$ activity against enteric pathogens**

| Enteric pathogens | NaCl (%) | | | pH | | | |
|---|---|---|---|---|---|---|---|
| | **0.5** | **1.0** | **2.5** | **3** | **5** | **7** | **9** |
| S. aureas | 15* | 16 | 12 | 13 | 11 | 10 | 9 |
| P.aeruginosa | 12 | 10 | 9 | 13 | 9 | 8 | R |
| E. coli | 20 | 16 | 10 | 13 | 10 | 9 | R |
| E. cloacae | 25 | 20 | 10 | 20 | 19 | 16 | R |
| S. dysenteria | 13 | 10 | 8 | 18 | 17 | 15 | R |

*Inhibition zone (mm) by using agar plate

The $BLS_{11}$ preparations from A.hydrophila 11 strains of A. hydrophila were tolerant to all three treatments of surfactant,and gave high activity against other genera of bacteria (enteric pathogen).The best result obtain with TritonX-100 against all bacterial strains especially for E. coli and E. cloacae with inhibition zone 33mm and 32mm respectively as shown in (Table 5).We suggested that the surfactants used effect on cell membrane which lead to BLS release, which induced wide clarity of zones of growth inhibition. In the other hand effect of organic acid on BLS production ($BLS_{11}$) has been studied and showed no remarkable difference in zone of inhibition when used acetone as effecter element, while both of isopropanol and ethanol have narrow inhibition zone range when compared with control strain. As well as chloroform and methanol have been tested and showed no effect on BLS production. Organic solvent did not inhibit the activity of the bacteriocin, which might confirm the presence of lipid moieties in the bacteriocin structure [29]

**Table 5: Effect of organic solvents, surfactants on $BLS_{11}$ activity against enteric pathogens**

| Enteric pathogens | Organic solvents | | | | | Surfactants | | |
|---|---|---|---|---|---|---|---|---|
| | Isopropanol | Acetone | Chloroform | Ethanol | Methanol | SDS | TritonX-100 | Tween 80 |
| S. aureas | 12 | 20 | R | 9 | R | 15 | 12 | 11 |
| P.aeruginosa | 11 | 12 | R | 11 | R | 8 | 11 | 11 |
| E. coli | 11 | 15 | R | 9 | R | 9 | 33 | 30 |
| E. cloacae | 13 | 11 | R | 9 | R | 11 | 32 | 30 |
| S. dysenteria | 15 | 22 | R | 15 | R | 14 | 25 | 18 |

All the experiments have been carried out twice, and all analyses have been carried out in duplicate.

In several pathovars, plasmid-born virulence or other traits have been reported [30]. In order to study the relationship of BLS of A. hydrophila strains and their indigenous plasmids , five selected A. hydrophila produced BLS have wide range effect on same species and enteric pathogenic stains were profiled are shown in (Table 6). All selected local isolates of A. hydrophila (3 isolates) harbored one plasmid except $BLS_{11}$ have two plasmids and $BLS_3$ have no plasmid content.

**Table 6: Plasmid number for selected** A. hydrophila

| A. hydrophila | Plasmid number |
|---|---|
| $BLS_1$ | 1 |
| $BLS_3$ | 0 |
| $BLS_5$ | 1 |
| $BLS_{10}$ | 1 |
| $BLS_{11}$ | 2 |

These results indicated that most A. hydrophila may be harboring plasmid mediated BLS, and there are no relation between BLS production and number of plasmid bands present in bacteria. In [31] showed that extra chromosomal analysis showed the presence, in 70% of the strains, of one to five plasmids with molecular masses ranging from 2.1 to 41.5 MDa, but it was not possible to relate this result with BLS production.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| A.hydrophila | Aeromonas hydrophila |
| BLS | Bacteriocin-like Substances |
| BHI | Brain Heart Infusion |
| PG | Peptone Glyserol |
| S.aureus | Staphylococcus aureus |
| P.aeruginosa | Psudomonas aeruginosa |
| E.coli | Escherichia coli |
| E.cloacea | Enterobacter cloacae |
| S.dysenteria | Shigella dysenteria |
| IA | Isoinhibitory Activity |
| HA | Heteroinhibitory Activity |
| DC | Deoxycholate-Citrate agar |
| MC | MacConkey agar |
| SBA | Sheep Blood agar |
| MRS | de Man Rogosa and Sharpe media |
| K2HPO4 | Potassium di hydrogen phosphate |
| MgSO4·7H2O | Magnesium Sulphate Dihydrate |
| MnSO4·4H2O | Tetrahydrated manganese sulphate |
| NaCL | Sodium Chloride |
| NaOH | Sodium Hydroxide |
| HCL | Hydrochloric Acid |
| TSB | Tryptone Soya Broth |
| SDS | Sodium Dodecyl Sulfate |
| BA | Blood agar |

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# دراسة تاثير العوامل الفيزيائية والكيميائية على انتاجية البكتريوسين من عزلات Aeromonas hydrophila

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## الخلاصة:

عزلت بكتريا Aeromonas hydrophila بنسبة ( 5.6%) من المصابين بألتهابات المعدة والأمعاء الحاد .بينما عزلت 19 سلالة معوية مرضية اخرى من المصابين بالأسهال وبنسبة (94.4%) .أن بكتريا A.hydrophila شائع عزلها من الاطفال اللذين يعانون من الاسهال الحاد ،أذ اظهرت النتائج ان 18 (78.26%) عزلة من A.hydrophila وجدت لدى الاطفال اللذين تقل اعمارهم عن 10 سنين ،موزعة بين 10 (43.47 %) ذكور ،8 (34.78%) اناث ،5( 21.73 %) بالغين. من جانب اخر لوحظ تكرار عزلها من الذكور14 (60.86 %) مقارنة بالاناث 9( 14 .39%). ستة سلالات من A.hydrophila لوحظ لديها فعالية البكتيريوسين ضد اثنا عشر من مجموع ثلاث وعشرون عزلة من البكتريا نفسها وكذلك على انواع بكتيرية اخرى: S.dysenteria , E.cloacea, E.coli , P.aeruginosa , S.aureus .وقد وجد أن البكتيريوسين المفرز من العزلة رقم 11 يمتلك فعالية تثبيطية على عشرة عزلات مختلفة لنفس البكتريا وفعالية تثبيطية على كل العزلات المرضية المستخدمة بالدراسة ، بينما البكتيريوسين المفرز من العزلة رقم 5 اظهر فعالية تثبيطية على اثنان من A.hydrophila وفعالية تثبيطية على البكتريا السالبة لصبغة كرام ، اما البكتيريوسين المنتج من العزلتين رقم 3 و 12 اظهر فعالية تثبيطية على عزلة E.coli فقط ولم يظهر البكتيريوسين المنتج من العزلة رقم 1 والعزلة رقم 10 اي فعالية تثبيطية على A.hydrophila او الانواع المرضية الاخرى. لقد تم استخدام الوسط الزرعي السائل (BHI) والذي اظهر اكبر انتاج بالمقارنة مع الاوساط الزرعية السائلة الاخرى مثل (PG) و ( الوسط المغذي السائل) والتي اعطت حصيلة قليلة من النمو. لقد تم اختيار البكتيريوسين المنتج من قبل العزلة رقم 11 وذلك لفعاليته الواسعة على نفس الانواع والسلالات المعوية المرضية الاخرى لدراسة تأثير الظروف الفيزيائية والكيميائية على انتاج البكتيريوسين من قبل A.hydrophila .ان البكتيريوسين المنتج من قبل عزلة A.hydrophila رقم 11 تحمل المعاملة بالمواد ذات الفاعلية السطحية ، اما تأثير الاحماض العضوية على انتاج البكتيريوسين من العزلة رقم 11 فقد ظهر عدم وجود اختلافات ملحوظة في قطر منطقة التثبيط عند استخدام الاسيتون كعامل مؤثر وقد اعطى الكحول الازوبروبانول والايثانول مناطق تثبيط صغيرة عند مقارنتها مع عزلات السيطرة .وقد اشارت النتائج الا ان معظم عزلات A.hydrophila تمتلك بلازميدات تشفر لانتاج البكتيريوسين ولا وجود لعلاقة بين انتاج البكتيريوسين وعدد حزم البلازميدات الموجوده في البكتيريا.