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Research Article

EVALUATION OF ANTICANCER PROPERTY OF BACTERIOCIN PRODUCED FROM *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

The study was carried out to check the anticancer property of bacteriocin produced from bacteria. In this research, isolation of strain of *Staphylococcus aureus* was done from skin swab samples and identification of strain was done by morphological and biochemical analysis. Further, adsorption-desorption method was used for the production of bacteriocin from isolated strain and SDS-PAGE analysis revealed its proteinaceous nature. We have performed HPLC for the purification of bacteriocin. MTT and Annexin V assay depicted anticancer property of purified bacteriocin. It has been found that bacteriocin has significant antitumor activity against MDA MB 231 cell lines.

Keywords: Anticancer activity, *Staphylococcus aureus,* Cell lines, Bacteriocin.

1. INTRODUCTION

Bacteriocins are antimicrobial peptide produced extra-cellularly by a number of Gram positive as well as Gram-negative bacteria. A well-known Scientist Gratia first discovered bacteriocin from *Escherichia coli* in 1925. Bacteriocins are proteinaceous substances having multifunctional capabilities and with high potential of antimicrobial activity. These molecules expressed anti-microbial activity against pathogenic and deteriorating bacteria, which justifies their role as biotechnological weapon. [1]. Bacteriocin has low toxicity and high potency which exist in both narrow as well as broad spectrum [2]. Cancer cells are diversified from the normal growth regulating mechanisms. In the case of normal cells, the cell renewal rate is found to be equal to cell death hence the production of new cells is regulated and there is stability in the number of a particular cell type. But cell did not respond to normal growth mechanism if inherited genetic mutations or environmentally induced mutations took place which lead to the production of cell clone that ultimately lead to the generation of tumor or neoplasm [3].

Nowadays, numerous techniques such as surgery, radiation therapy, and chemotherapy are available for cancer treatment, the most preferable treatment is chemotherapy, but there are limitations of this therapy including damage of healthy cells also. Another constraint is that these cancer cells frequently become resistant to the chemotherapeutic drug. Therefore, there is an immediate requirement of effective cancer therapies [4]. Bacteriocins are found effective against cancer cells even at nano and micromolar concen-tration. The structure of bacteriocin determined the potential and competency of bacteriocin to affect the cancer cells. It depends on the number of positively charged amino acid, present, hydrophobicity, strength for the formation of amphipathic structures or oligomerization [5]. The increased negatively charged surface of cancer cells makes them more exposed to the cytotoxicity of the bacteriocin. Most of bacteriocin causes both apoptosis and necrosis [6]. There are several methods that are designed to perform cancer therapy with the help of peptides and proteins as well as to combat the antimicrobial infections. The combination of peptides, protein and conventional drugs can be used for this purpose [7-9]. The formation of such heterologous compound combination with peptide and protein helps in the site directed activity [10, 11].

2. MATERIAL AND METHODS

2.1. Isolation and Identification of *Staphylococcs aureus*

Staphylococcus aureus strain was isolated using selective media: Mannitol salt agar and identified following burgey's manual. DNase Test and Coagulase test were performed for the further confirmation of species [12, 13].

2.2. Production of crude Bacteriocin

For the production of bacteriocin, 24 hr overnight culture of *S. aureus* was taken and centrifuged at 10,000 rpm for 10 minutes at 4°C. The pellet was discarded and the supernatant was used as crude bacteriocin [14].

2.3. Assay for bacteriocin production

The affectivity of the bacteriocin produced was determined using a well-known Agar well diffusion assay [15]. MHA plates were prepared and inoculated with indicator strains by spread plate technique and plates were labeled properly. Crude bacteriocin prepared was added into each well. The plates were incubated at 37°C for 24 hrs. After completion of incubation, a clear zone of inhibition was observed.

2.4. SDS PAGE Analysis and Purification of Bacteriocin

SDS PAGE was used to analyze the proteinaceous nature of antimicrobial peptide as well as size estimation. The analysis of bacteriocin was performed with 12% resolving gel and 4% stacking gel. For the estimation of molecular size of bacteriocin, Coomassie brilliant blue R-250 was used to stain the gel**.** High performance liquid Chromatography (HPLC) was used to purify the bacteriocin. Mobile phase used was acetonitrile and HPLC grade water containing 0.1% Trifluoroacetic Acid (TFA). C-18 column was used for sample loading and separated by a linear biphasic gradient of 20 to 80% acetonitrile over 30 minutes at a flow rate of 0.5 ml per minutes.

2.5. Mode of Action

For the detection of mechanism of action of bacteriocin, potassium ion release from the target cells was measured by flame photometer [16]. The target strain *Listeria monocytogenes* were cultured to mid-log phase and recovered. Tris-acetate buffer (10 mM, pH 7.4) consisting of 100 mM NaCl was used to wash the cell thrice. Afterwards, re-suspension of cells in the same buffer was done. The bacteriocin was added with varied concentration to the target strain. Control was taken without adding the bacteriocin in it. The release of K^+ ions was monitored at the different concentrations of bacteriocin and calibration was done using KCl solutions (40 and 100ppm).

2.6. Anticancer property of bacteriocin

2.6.1. MTT Assay

The cytotoxicity activity of the test sample bacteriocin was evaluated against MDA MB 231 cell lines using MTT assay [17]. 96-wells plates were seeded with MDA MB 231 (10,000 cells/well) and 200µl of DMEM media afterwards, overnight incubation was done for the attachment of cells. After the cell attachment the aspirated media and 200µl of fresh media consisting of bacteriocin and doxorubicin (anticancer drug) were added at a concentration of 1, 5, 10, and 20µg/ml and further incubated for 24hrs. After the completion of incubation, the media was aspirated and 200µl of DMSO used for the solubilization of formazan crystals and the optical density (OD) was measured at 550 nm using an ELISA plate reader (BioTek, USA). The cell viability was evaluated by

Relative cell viability = Absorbance of test sample/ Absorbance of a control sample

2.6.2. Annexin V assay

The observed results of cytotoxicity were further confirmed by the apoptosis assay [18]. 10 µM of bacteriocin was incubated with MDA MB 231 cells for 4 hrs. After completion of incubation, HBSS was used to wash the cells three times and stained with 6-carboxyfluorescein diacetate (6-CFDA) and Annexin V-Cy3.18 conjugate (AnnCy3) and observed under the red and green fluorescent channel, by CLSM. The cells stained with red, green, and yellow (overlap of red and green) fluorescence were considered as necrotic, live, and apoptotic, respectively. Afterwards, the quantitative analysis of apoptosis was also estimated by calculating ratio of the red and green fluorescence intensity known as the Apoptosis index. The fluorescence intensity was quantified by Image J software (U. S. National Institutes of Health, USA). [19].

2.7. Statistical Analysis

The level of statistical significance was estimated as p value ($p = <0.05$) using one way ANOVA. Where necessary experiments were performed in triplicate and mean values were plotted.

3. RESULTS AND DISCUSSION

Out of 93 skin swab samples, 17 isolates confirmed as *Staphylococcus spp*. Further, coagulase test and DNase test confirmed *Staphylococcus aureus*. After confirming the strain, cell free supernatant was produced at 10,000 rpm for 30 minutes and antimicrobial activity was checked against indicator strains of *Listeria monocytogenes*. Out of 17 cultures, 6 isolates showed a clear zone of inhibition ranging from 14 mm to 28mm against indicator strain. Isolate 4 showed significantly higher zone of inhibition (28mm) followed by isolate 5 (26 mm) and isolate 2 (22 mm). Isolate 1 and isolate 6 showed minimum zone of inhibition 20 mm amd 16 mm respectively. Isolate 3 showed lowest Zone of inhibition 14mm (fig.1, table 1). Similar results were observed from another study showed bacteriocins synthesized from *Lactobacillus spp*. causes bactericidal effect on Foodborne bacteria [20].

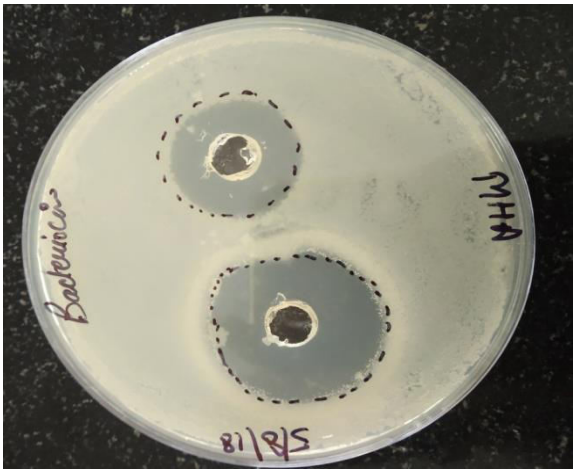


Fig. 1: Zone of inhibition against *Listeria monocytogenes*

Table 1: Bacteriocin activity against *Listeria monocytogenes*

Sample no.	Zone of Inhibition (mm)
S- 7	20 ± 0.2
S- 21	22±0.5
S – 23	14±0.3
S – 36	28± 0.5
S – 42	26± 0.4
S – 49	16±0.3

The SDS PAGE confirmed the proteinaceous nature of the elute with molecular mass obtained less than 10kDa. (fig.2). High performance liquid Chromatography (HPLC) was used to purify the bacteriocin and it gave a single peak at 5 min time interval of intensity at an absorbance of 280nm. Retention time was 5.3 at 280 nm absorbance.

Flame photometry analysis depicted bacteriocin causes pore formation into the cytoplasmic membrane of indicator strain as the potassium ion efflux has been observed in the time interval of 1 min (table 2, fig. 4).

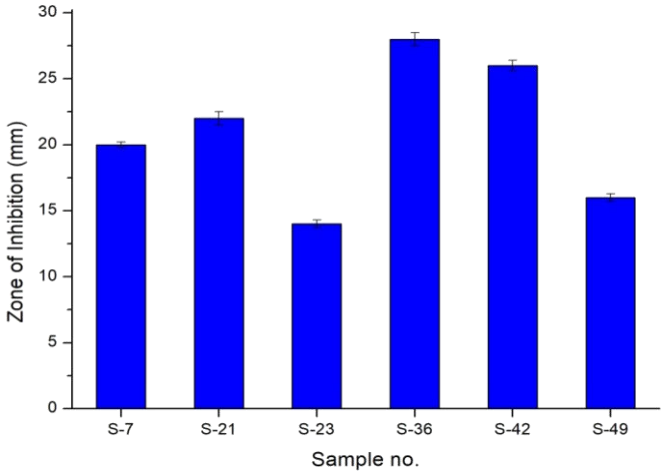


Fig. 2: Bacteriocins activity against *Listeria monocytogenes*

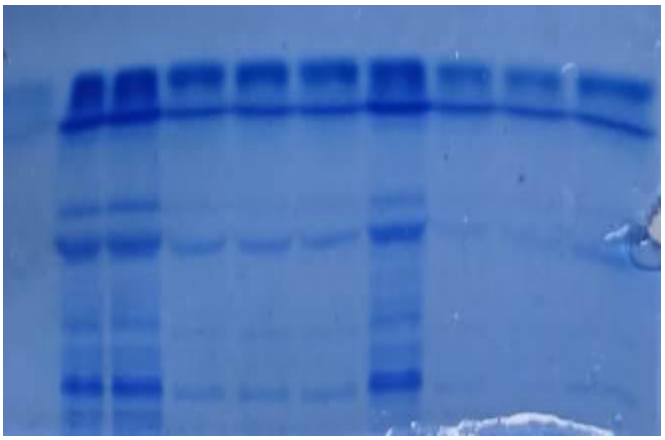


Fig. 3: SDS PAGE

Table 2: Showing efflux of K^+ from cytoplasmic membrane of indicator strains

Time interval (min)	K^+ efflux (ppm) (*Listeria monocytogenes*)	K^+ efflux (ppm) Control
1	16	0.5
2	16	0.5
3	15	0.5
4	12	0.5
5	10	0.5

The cytotoxicity activity of the test sample bacteriocin was tested against MDA MB 231 cell lines using MTT assay. The IC_{50} value of bacteriocin was found to be 18.026 ± 0.067(µg/ml) in comparison to IC_{50} value of 5.557±0.085 (µg/ml) of doxorubicin (an anticancer drug). The observed results of cytotoxicity were further confirmed by the apoptosis assay. The apoptosis index of bacteriocin was found to be 0.50. Both activities displayed that the test sample bacteriocin has significant antitumor activity against MDA MB 231 cell lines (table 3, fig. 3). Al-Madboly *et al.* observed the highest anticancer effect of bacteriocin against HepG2 cells [21].

Table 3: Certance of cell viability at different concentration

Con.(µg/ml)	Percentage cell viability of Bacteriocin	Percentage cell viability of Doxorubicin	Mean	Std Dev
1	98.14±1.11	90.88±2.70	94.51	5.13
5	90.88±2.70	65.33±1.79	78.11	18.07
10	65.33±1.79	35.97±2.60	50.65	20.76
20	49.36±2.13	18.67±2.63	34.02	21.70

F value = 4.69, P value = 0.0848, Level of Significance <0.05

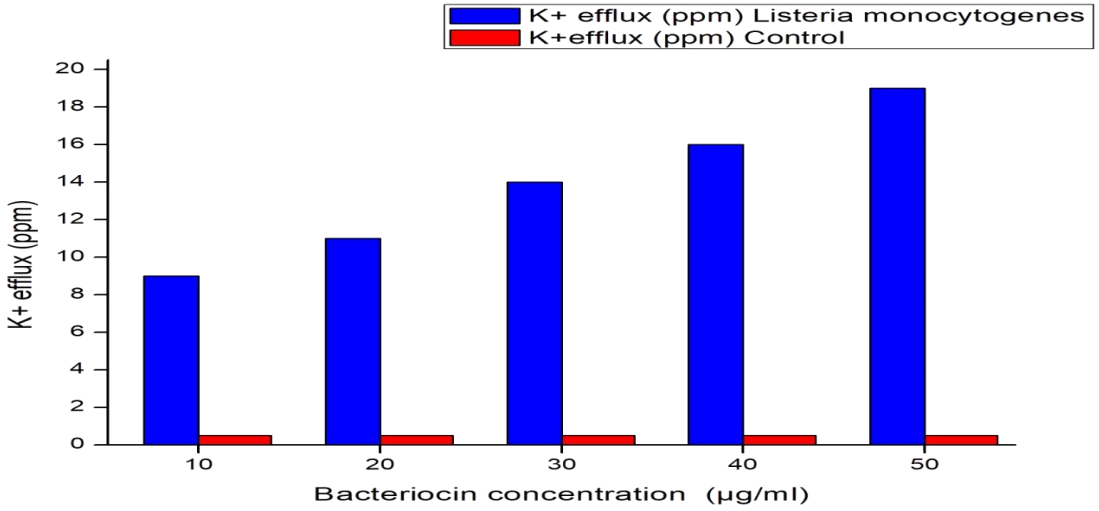


Fig. 4: Showing efflux of K^+ from cytoplasmic membrane of indicator strains (*Listeria monocytogenes*)

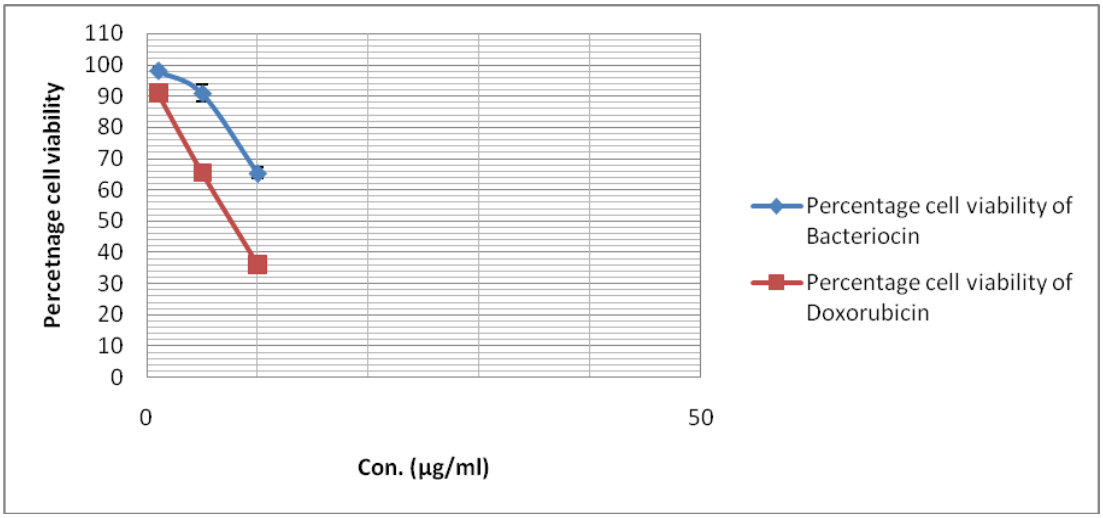
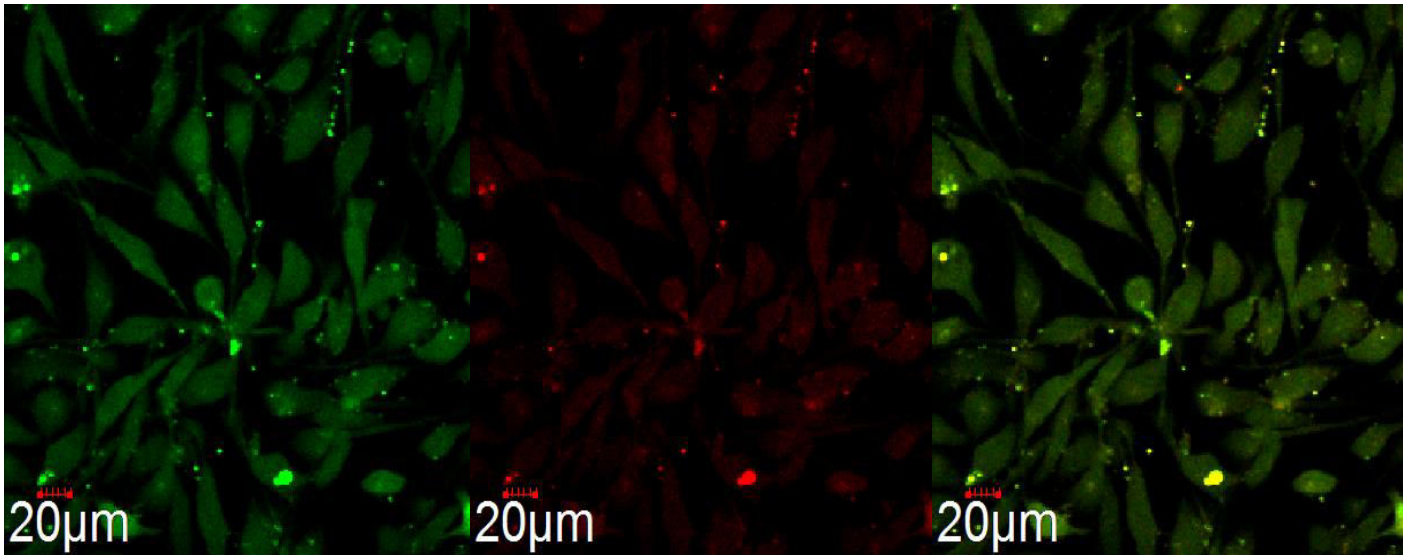


Fig. 5: Linear curve between Percentage cell viability and concentration



(A) green channel depicts the fluorescence from carboxyfluorescein (cell viability marker dye); (B) red channel depicts fluorescence from Annexin Cy3.18 conjugate (cell apoptosis marker dye) (C) represents the overlay image of a panel (a) and (b)

(A) (B) (C)

Fig. 6: CLSM image of apoptosis assay

4. CONCLUSION

In the present investigation, it was found that bacteriocin produced from *Staphylococcus aureus* showed remarkable anticancer properties against MDA MB 231 cell lines. The IC_{50} value of bacteriocin was found to be 18.026±0.067 (µg/ml) in comparison to IC_{50} value of 5.557±0.085 (µg/ml) of doxorubicin.

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Conflicts of interest

The author(s) declared no potential interest with respect to research, authorship and/or publication of this article.

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