



DETERMINATION OF BACTERIOCIDAL ACTIVITY OF BACTERIOCIN PRODUCED BY A CLINICAL ISOLATE OF *E. COLI* AGAINST HUMAN PATHOGENS

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ABSTRACT

Aim: The aim of this study is to estimate the bactericidal activity of bacteriocin against the common human pathogens. A total of 62 specimens were collected from patients attending the department of surgery, Karpaga Vinayaga Institute of Medical sciences and Research Centre, during a period of six months.

Methodology: Specimens collected were superficial pus swab from the wound, deep tissue biopsy and scraping from the base of the ulcers. Samples were cultured on Blood agar (BA) and MacConkey agar (MA). The plates were incubated at 37C over night. The aerobic organisms were identified by standard microbiological procedures as described in Bailey and scott's Daignostic Microbiology. (Finegold et al.1986).(1) The E coli which was isolated and identified was used in the preparation of crude bacteriocin.(2) It was purified by Ammonium sulphate precipitation(3)and concentrated by centrifugation. Desalting of protein was done by dialysis method.(4) The protein concentrations of these purified bacteriocin was determined by Lowry's method.(5) Bacteriocin prepared from E coli was subjected for its bactericidal activity against some human pathogens by minimum inhibitory concentration method. Optical density (OD) of the culture was measured at 660 nm using spectrophotometer. Bactericidal activity of bacteriocin against human pathogens was estimated.

Results: The results were analyzed, most of the pathogens need a very low concentration of the bacteriocin for the minimum inhibitory concentration (MIC).We observed that there is a slight variation in MIC among the different bacterial pathogens.

Conclusion: In General when the concentration of the bacteriocin increased then the OD values of the culture tubes were decreased. In conclusion Bacteriocin not only have the bacteriocidal activity but also dose dependent in different bacteria.

Key Words: Bacteriocin, Bactericidal activity, Microbial virulence

INTRODUCTION

Pathogenic microbes produce disease in man is due to certain enzymes and toxic substances released by bacteria called virulence factors. Some chemicals released by the bacteria may cause self destruction are known as autolytic enzymes. Similarly some other biologically active compounds, produced by the bacteria may active against strains of other species or closely related species. This type of bactericidal agents produced by Gram negative bacteria are called bacteriocins.(6) Bacteriocins are ribosomally synthesized antimicrobial peptides.(7) Classification of bacteriocins based on molecular size, producer organisms, chemical structure and the mode

of action. Various names are given to these agents such as colicin, pyocin and or marcescins etc. Bacteriocin affect the essential function of livings cells such as transcription, replication and cell wall biosynthesis.(8) They also act by forming membrane channels or pores that destroy the energy potential of sensitive cells.(9) However bacteriocin producing strains are resistant to their own product.

It has been evaluated for intended use as mouth wash since it kills the bacteria which cause dental plaque. Hence it is safe to use as a natural alternative to antibiotics and chemical germicides. Keeping this in mind, it was planned to study the bactericidal activity of bacteriocin on some of the human

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pathogens. Informed consent was obtained from those who underwent the test procedure in this study. Ethics committee of Dr. MGR University approved this study.

MATERIALS AND METHODS

A total of 62 specimens were collected from guttural, thigh, knee, leg, foot, breast and umbilical areas which included the wound swabs, pus, tissue biopsy and scraping from ulcers. Samples were collected aseptically from patients attended the surgery department of Karpaga Vinayaga Institute of Medical science and Research Centre, Chinnakolambakam. Samples collected were inoculated on to culture media and the organisms grown after incubation were identified by standard microbiological methods. Among the organisms isolated, the E. coli was the most commonly observed (10). The clinically isolated E.coli was used in the preparation of crude bacteriocin, because it is the common source of bacteriocin. (11) E. coli isolates had been grown on nutrient broth with 5% inoculums by batch fermentation at a pH of 6.2. The broth culture was incubated at 37c for 12 hours. For the separation of bacteriocin the culture was centrifuged at 8000 rpm for 20 minutes. Cell free supernatant was subjected to solid ammonium sulphate at pH 4 with gradual increase of 20 to 90% saturation and held for 3hours at 4c. The mixtures were centrifuged at 12000 rpm for 20 minutes. The precipitate was recovered and it was then suspended in 5ml of 50m.mol of sodium phosphate buffer at pH 7 followed by desalting of the protein by dialysis. Protein concentration was determined by lowry's method and compared with the standard bovine serum albumin.

Estimation of bacteriocin activity

The purified bacteriocin compound from *E.coli* was subjected for its bacteriocidal activity against common human pathogens. Organisms used for the assay were *Klebsiella spp*, *Salmonella typhi*, *staphylococcus aureus* and *corynebacterium*. Estimation of MIC for the above culture of organisms, 0.5 ml of each log phase cultures were transferred in to test tubes contain nutrient broth of appropriate concentration of bacteriocin. 100µl/mL to 500µl/ml were prepared and added to 8ml of broth culture and were inoculated at 37c for 24hours. Minimum inhibitory concentration was estimated by measuring the optical density of the culture tubes at 660nm using spectrophotometer.

RESULTS

Antibacterial sensitivity of bacteriocin against human pathogens were estimated and the results were tabulated .Table: 1 showed the turbidity measurements of *klebsiella spp*

Table 1: Turbidity measurements of *klebsiella spp*

Tube No	Bacteriocin concentration(µl/mL)	ODvalues (660nm)
1	100	0.06
2	200	0.04
3	300	0.02
4	400	0.01
5	500	0.00

Klebsiella spp showed a gradual variation in the turbidity values observed in the optical density. The values depend on the concentration. The concentration and optical density were inversely proportion. When the concentration increases the OD value decreases. As there was no growth in the last tube (500 µl/mL) indicated that *klebsiella spp* was sensitive to bacteriocin.

Table 2 showed the turbidity measurement of *Salmonella typhi*.

Table 2: MIC of bacteriocin in *Salmonella typhi*

Tube No	Bacteriocin concentration(µl/mL)	OD values (660nm)
1	100	0.06
2	200	0.05
3	300	0.04
4	400	0.02
5	500	0.01

In the case of *S.typhi* turbidity measurement was recorded a gradual variation in the OD values again it depended on the concentration of bacteriocin. However the MIC was not achieved since the OD value is not showed 0.00 even at 500 µl/mL

Table 3 showed the turbidity measurement of *Staphylococcus aureus*

Table 3: MIC of bacteriocin in *Staphylococcus aureus*

Tube No	Bacteriocin concentration (µl/ mL)	OD values (660nm)
1	100	0.04
2	200	0.03
3	300	0.02
4	400	0.01
5	500	0.00

Results of MIC determination in *Staphylococcus aureus* showed a regular decrease in the OD values as the concentration increased and there was no growth at the highest

concentration 500 µl/mL. There was no growth therefore no turbidity.

Table 4 showed the turbidity measurement of *Corynebacterium diphtheriae*.

Table 4: MIC of bacteriocin in *Corynebacterium diphtheriae*

Tube No	Bacteriocin concentration(µl/mL)	OD values (660nm)
1	100	0.00
2	200	0.00
3	300	0.00
4	400	0.00
5	500	0.00

It was observed a different type of result when doing the MIC determination of *Corynebacterium diphtheriae* even at the lowest concentration (100µl/mL) the OD value showed 0.00, suggested that the strains were highly sensitive to bacteriocin.

DISCUSSION

Bacteriocins are the subject of increasing attention as a major replacement for the conventional antibiotics and as natural food preservatives.(12) There are multiple bacteriocins present in the population of *E.coli* (13) and (14). Colicin production and the effect of growth inhibition was tested by solid methods.(15) In the present study turbidometry was used to assay the growth inhibition. In medicine the bacteriocins are important as they are produced by some non pathogenic bacteria act as commensal and loss of these bacteria following the use of antibiotics may cause the invasion of opportunistic pathogens. Hence it is decided to demonstrate the effect of bacteriocin on human pathogens. Further most of the mechanisms of bacteriocin in killing the bacteria are similar to that of antibiotics.(16)(17) Therefore it may replace the use of the antibiotics in the treatment. Hence this study was conducted as a step to determine the effect of bacteriocin on human pathogens.

CONCLUSION

Though MIC studies of bacteriocin on human pathogens may not showed any consolidated report, but the results were encouraging. Present study results revealed that some strains

of bacteria were sensitive to bacteriocin and are showed a response to its concentration. Each of the pathogen responded differently. Further studies are essential with still highly purified form of bacteriocin and the pathogens to be studied with wide range of bacteriocin concentration.

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