Inhibitory Activity of Staphylococcal Bacteriocin Against Pathogenic Bacterial Strains

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Abstract—The objective of the study was to determine the inhibitory activity of bacteriocin isolated from staphylococcus aureus towards some pathogenic bacterial species. The inhibitory activity of bacteriocins produced by Staphylococcus aureus was tested against pathogenic bacterial strains of Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus feacalis and Proteus spp. A total number of 30 samples were collected from food waste (bread, rice, vegetables). Among them 8 samples showed positive bacterial growth. After performing the morphological, culture characteristics and biochemical reactions, 3 bacterial species among the 8 were identified as Staphylococcus aureus. The 3 staphylococcus aureus were marked as Staph 1, Staph 2 Staph 3 respectively. Bacteriocin production by staphylococcus aureus (staph 1) was observed when Staph 1, Staph 2 and Staph 3 were tested for antimicobial sensitivity by using well assay against indicator pathogenic bacterial strains. Pseudomonas aeruginosa showed maximum sensitivity towards staph 1 whereas Klebsiella pneumoniae showed resistance. The optimum growth condition of the bacteriocin production was observed in pH 8 and 35oC after overnight incubation. The bacteriocin from staph 1 can be further purified and this can be used in treatment of pyogenic infections caused by pseudomonas aeruginosa.

Keywords: Staphylococcus aureus, Bacteriocin, Pyogenic infection, Food waste.

1. INTRODUCTION

A bacteria can inhibit the growth of another is a well known fact which happens because of substances produced, excreted or secreted by a bacteria Bacteriocins belong to this class of substances too [1,2]. They are proteinaceous substances which inhibit their inhibitory antibacterial action on closely related species of bacteria Their mechanism of action involves binding to specific receptor sites followed by action on the cell . Staphylococcal bacteriocins are broad spectrum substances which are active against both gram positive and gram negative bacteria [3,4]. However it has been noticed that bacteriocins produced by gram positive bacteria never act on gram negative organisms. Because bacteriocins act on bacterial species closely related to their parent bacteriocins, it has been debated that bacteriocin producing bacteria are ecologically more evolved and superior the bacteriocins that don't produce bacteriocins [5,6].

2. METHODOLOGY

2.1. Microbial Study

A total of 30 samples were collected from food waste including bread, rice and vegetables from our daily use from March to May, 2015. Microbiological culture was done by doing serial dilution of those samples and then pour plate technique. Out of 30 samples 8 samples were found to be bacteria positive. Among those 8 positive culture 3 were found as S. aureus by observing Gram staining, culture characteristics and biochemical reaction results [7]. The three staphylococcus aureus isolates were marked as Staph 1, Staph 2 and Staph 3 respectively.

2.2. Antibiotic sensitivity Test

The antimicrobial sensitivity test was done for Staph 1, Staph 2 and Staph 3 by using antibiotics discs Vancomycin, Clindamycin, Mupirocin, Cefepime, Telavancin, Methicillin, Tetracycline, Ceftaroline, Daptomycin and Oxazolidinones. The antibiotic susceptibility test was performed by Modified Kirby - Bauer method. The Staphylococcal colonies were suspended into peptone water medium and incubated for 2 hours. Mueller - Hinton agar plates were carpeted with this suspension with the help of cotton swab stick. After that antibiotic discs were transfered on this medium and the culture plates were then incubated overnight at 37° C [8]. The Zone of inhibition were measured.

2.3. Examination of the Efficient Strain Producing Staphylococcal bacteriocin

All 3 staphylococcus isolates were selected for investigation of the efficient strains producing bacteriocin by well diffusion method [9] as they were sensitive to most antibiotics against some pathogenic bacterial strains of *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus feacalis* and *Proteus spp* (collected from Primus Laboratory, Chatterpur, New Delhi). To determine the most bacteriocin producing strain 5 nutrient agar plates were taken and were inoculated with 100 micro liter of each pathogenic bacterial strains (0.5 McFarland stand) after incubating them in nutrient broth medium. Staph 1, Staph 2 and Staph 3 were grown in MRS broth for 48 hrs in different pH and temperature. The broth tube were then centrifuged at 5000 rpm for 10 minutes to seperate cells. Three wells of 6mm each were cut inside every pre inoculated nutrient agar plates and 100 micro litter of supernatant fluids of staph 1, staph 2 and staph 3 were poured inside the wells and incubated at 37° C for 24 hours. Zone of inhibition were measured and recorded.

3. RESULTS AND DISCUSSIONS

Identification of staphylococcus aureus were confirmed by catalase, coagulase and IMViC tests. They showed beta hemolysis on blood agar medium. All of 3 staphylococcus aureus isolates were tested with 10 different antibiotics in our study. All isolates were sensitive to vencomycin with ratio (100%) and Methicillin (74.2%). And they were resistant with ratio Tetracycline (55.2%), Clindamycin (60.46%), Cefepime (72.4%), Oxazolidinones (76.8%), Daptomycin (88.3%), Ceftaroline (90.24%), Telavancin (93.8%) and Mupirocin (100%). Staph 1 shows most bacteriocin production at 350 C and pH 8. Pseudomonas aeruginosa was highly sensitive towards bacteriocin Staph 1 where as streptococcus pyogenes, Enterococcus feacalis and Proteus spp were moderate sensitive and Klebsiella pneumoniae was resistant to bacteriocin of Staph 1. No bacteriocin production was found in Staph 2 and Staph 3. Bacteriocins are regular antimicrobial specialists delivered by Gram positive microbes. BLIS have potential applications against an extensive variety of human and creature illnesses. They are ribosomally integrated antimicrobial peptides; they are deadly to microbes firmly identified with the creating microscopic organisms.the recent being ensured by a safety wonder [10]. Besides, since bacteriocins, created by microscopic organisms, are thought to have a vital part in building up the biological system [11]. The bacteriocin displayed here may be in charge of a piece of the control instrument of microbial biology. Bacteriocins are found in each bacterial species inspected to date [12]. Bacteriocins have far reaching applications in epidemiological studies particularly as markers for microscopic organisms.

From our study we came to the conclusion that the bacteriocin from staph 1 can be further purified and this can be used in treatment of pyogenic infections caused by pseudomonas aeruginosa.

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