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Efficacy Evaluation and Partial Characterization of Methicillin Resistant *Staphylococcus aureus* (MRSA) Sensitive Bacteriocin Producing *Lactobacillus*

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Abstract—*In the present system of healthcare antibiotics play an important and compulsory role in infection control and ensuring well being of the individual and community. But the injudicious use of antibiotics has led to development of antibiotic resistance and issues such as nosocomial infection etc. Methicillin resistant staphylococuus aureus (MRSA) is one well known much researched example of antibiotic resistance. Bacteriocins are a diverse group of proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain. In this study Bacteriocin producing lactobacillus was isolated and was found to be one of the therapeutic options that can be used to tackle MRSA infections.*

Keywords*: MRSA, Antibiotic resistance, Bacteriocin.*

1. INTRODUCTION

In the present day world Antibiotic resistance is one big reason because of which treatment of infectious diseases has become increasingly difficult. One of such strain is MRSA (Methicillin Resistant *Staphylococcus aureus*). It causes different infections from minor soft tissue damage to life-threatening diseses as well as toxic shock syndrome (1). Vancomycin was the only antibiotic to treat such kind of infection. But in 1990s, rising vancomycin resistant bacterial infection has become major challenge to physicians (2,3). Finding out new antimicrobials to treat such infections is a matter of grave importance. Lactic acid bacteria (LAB) are human friendly bacteria and are important for human well being. They colonises intestine where they invade harmful bacteria through different strategies. bacteriocin production is one of their defence mechanism.(4). Bacteriocins are protenecious toxins which are synthesized in bacterial ribosome having a narrow spectrum of antimicrobial activity to closely related baterial species. Presently some bacteriocins have been isolated and characterized by their spectrum of antimicrobial activity, gene sequence, molecular weight and biochemical characteristics (5). The present work deals with isolation of Lactobacillus strains from dairy food waste sources, screening of bacteriocin producing lactobacillus, partial biochemical characterization and illustration of antimicrobial efficacy of bacteriocin on MRSA (ATCC33591).

2. MATERIAL AND METHODS

2.1 Isolation of Lactic acid bacteria

Isolation of bacteria were done by pour plate technique in MRS agar plates (6). Samples (dairy food waste) were serially diluted and 50µl of each sample were placed in empty petriplates and then melted MRS agar were poured into those petriplates. After solidification plates were incubated at 37^0C for 24 - 48 hours to observe bacterial colonies.

2.2 Screening of Bacteriocin Producing Strains

MRs broth tubes were prepared (each tube containing 5 ml of MRS broth) and bacterial colonies were inoculated into each tubes and incubated for 24 hours at 37^0C. Turbidity was observed inside the tubes and from each tube 1.5 ml of isolates were transferred into eppendrof tube (2ml). The eppendrof tubes were centrifuged at 40^0C (1000 rpm) for 5 minutes. The cell free supernatant was collected (0.5ml) into another eppendrof tube and phosphate buffer (0.5ml) was added into each eppendrof tube for neutralization.These crude

bacteriocins (cell free supernatants) were used to check their antimicrobial activity against MRSA (ATCC33591) by well diffusion method described by Schillinger and Lucke (7).

2.3. Antimicrobial Activity of Lacticin against MRSA

MH agar media was used to determine antimicrobial activity. Each test tube containing 7ml of soft agar medium was prepared and add 50µl of MRSA (ATCC33591) young culture. Soft agar containing MRSA (ATCC33591) was poured above previously prepared MH agar medium. After solidification 6 mm wells were prepared inside medium using sterial borer. Cell free neutralized supernatants (100µl) were poured in each well. The plates were kept in a cool room for 4 hours and then incubated overnight at 37^0C for 18 hours to observe the zone of inhibition. The zone of inhibition was measured by a meter scale.

2.4. Enzyme Treatment for Identification of Bacteriocin

Cell free neutralized supernatants were treated with different types of enzymes like protease, pepsin, trypsin, chymotrypsin and proteinase K and incubated overnight to observe inhibition zones. The supernantan containing protenaceous compound was confirmed by observing disappearance of zones.

2.5. Identification of Bacteriocin Producing Culture

The Bacteriocin producing cultures were identified by culture characteristics, gram reaction and different biochemical reactions.

3. RESULTS

A total of 30 gram positive rods and catalase negative bacteria was isolated from diluted dairy food waste samples. Their cell free neutralized supernatant were treated with enzymes and only five of them ' 8FW, 12 FW, 15 FW, 28 FW, 29FW ' showed negative results (disappearance of clear zones) ensuring their proteinaceous nature. They were checked for their antimicrobial activity against MRSA. Bacteriocin of only one strain “28FW” exhibited zone of inhibition. The strain 28FW showed creamy white small colonies on MRS agar medium. Their optimum growth temperature is 35^0C - 38^0C. They can be grown at 45^0C but no growth is seen at 15^0C. Small round ended gram positive rods were seen under microscope after gram staining. Biochemical characteristics of strain “28FW” showed positive for different sugar fermentation like lactose, sucrose, maltose and mannose. They were negative for esculine hydrolysis and arginine hydrolysis. All these confirmations indicated that isolate belongs to Lactobacillus acidophillus groups.

4. DISCUSSION

It is an accepted fact that antibiotics occupy an important place in the field of medicines. These are used for treatment of humans and in agriculture for better livestock. Unfortunately, the beneficial effect of antibiotics has led to injudicious use. This is a major public health concern presently (8). This injudicious use of antibiotics has led to direct tons of antibiotics into the world in the last few decades (9). This has led to the appearance of resistant strains. One among them smethicillin-resistant S. aureus (MRSA). S. aureus causes serious nosocomial infections associated with considerable morbidity and mortality (10). MRSA, that are resistant to all lactam antibiotics and other antimicrobial classes makes infectious disease much more difficult to treat. There are several different determinants of severity of an infection like serious underlying diseases, period of hospitalization etc.Patients with MRSA infections and other similar infectionsi face more hardship in terms of morbidity and mortality than those infected with non resistant infecfions (11, 12, 13). Another disturbing statistic is the increased prevalence rates of MRSA from 2% in 1990 and 1991at England and Wales to a peak of 43% in 2002, with a small amount of decline thereafter (14). An interesting fact associated with MRSA is that only a few clones are spreading the world over with high resistant rates (15). This underlines the need of new methods for effective control over MRSA infections. The use of bacteriocins seems to be promising. They are presently used as food additives, Research is being conducted into role of bacteriocins as antimicrobial agents (16, 17). Because of their target specificity, bacteriocins may be more effective as compared to the regular antibiotics (18). Different groups of lactic acid producing bacilli (LAB) produce bacteriocins that have been subject of research for their ability to increase shelf life and to inhibit pathogenic bacteria in food products (17). LAB are also been shown to have a positive effect on symptoms associated with irritable bowel syndrome (19) and infant asthma (20) as well as decreasing the duration of the diarrhoea (21). LAB might be the ideal candidate in producing bacteriocin effective against MRSA. For this reason, we used the MRS media that is a selective media for LAB and isolated LAB from dairy food waste samples. Only one bacteriocin producer effective against MRSA we got among 30 isolates. Through culture charecteristics, morphology and biohemical reactions it was identified as lactobacillus acidophillus groups. (22, 23). This work reviles that Bacteriocin produced by L. acidophillus can be sensitive against MRSA. This could be beneficial for human wellbeing in long term future prospects.

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