Isolation and characterization of Vibriocin from marine environment

In recent years regulation authorities are more stringent about the use of synthetic preservatives in the food industry. On the other side the emergence of new type of pathogens continues in food leading to higher inciden-ces of food poisoning outbreaks. Hence, pre-venting the growth of pathogenic microorganisms is essential for food quality and safety. Most of the decontamination technologies such as cooking, pulsed light, high pressure, ozone, ultrasound processing etc. are not efficient to destroy the pathogenic bacteria and are not compatible with the delicate texture and flavour of seafood. Hence, a new technology that is gaining widespread attention is the bio-preservation technology. Bio-preservation implies inoculating the food with microorganisms, or their metabolites, which have potent antibacterial properties. One such bio-preservative approach is the use of bacteriocins. Bacteriocins are bacterial substances having an essential biological protein moiety and a bactericidal mode of action centred against other bacteria. For example, Nisin has already been given the status of a preservative by USFDA and is being used commercially in food industries. Hence, an attempt has been made in Mumbai Research Centre of ICAR-CIFT for the isolation and purification of Vibriocin, a bacteriocin from Vibrio species. Further, its efficacy in inhibiting the growth of major seafood spoilage and pathogenic organisms has been evaluated.

A total of 40 Vibrio bacteria isolated from clam samples were tested for antimicrobial activity (Fig. 1). The activity was tested against nine different pathogens such as *Aeromonas hydrophila, Bacillus cereus, B. subtilis, Escherichia coli, Listeria monocytogenes, Salmonella, Staphylococcus aureus, Vibrio cholerae* and *V. parahaemolyticus*. Only two strains of *Vibrio* (Isolate 7 and 8) were shown to produce a bacteriocin-like substance (Fig. 1). Isolate 7 showed potent antimicrobial activity against *Staphylococcus aureus* (Fig. 2), whereas Isolate 8 showed potent antimicrobial activity against *S. aureus* and *B. subtilis* (Fig. 3).

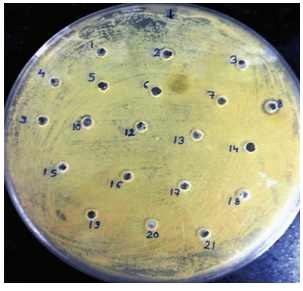

Fig. 1. Bio-screening of the isolates against *Bacillus subtilis* and the zone of clearance around isolate Number 8

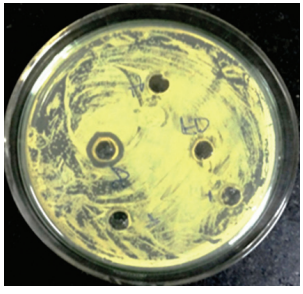

Fig. 2. Isolate 7 showing maximum activity against *S. aureus* after dialysis

The bacteriocin producing culture was centrifuged at 10,000 rpm for 10 minutes and the cell-free supernatant (CFS) was filter sterilized through 0.45µm syringe filter and tested for its activity under varying conditions such as pH, temperature and activity after proteolytic enzymes treatments. The bacteriocins retained their activity over a wide range of pH (4 to 8); however, maximum activity was observed at

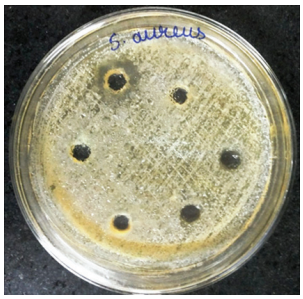

Fig. 3. Isolate 8 showing maximum activity against *B. subtilis* after dialysis

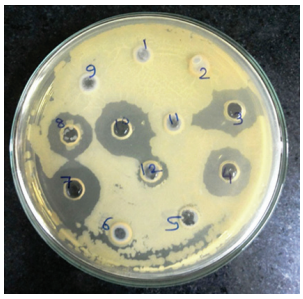

Fig. 4. Enhanced effect of Vibriocin after treatment with SDS at different pH (Well No. 3, 4, 7, 8, 10); untreated control (Well No. 12)

neutral pH. Bacteriocin possessed the maximum activity at a temperature of 40 °C. Even though, the activity was observed at 60 °C, it was lost on heating beyond 30 min. at 60 °C, which indicates that the bacteriocins are well adapted to the environment. The bacteriocins showed complete sensitivity to the proteolytic enzymes such as lysozyme, papain, proteases and proteinase K, which suggests that the Vibriocin is a protein and will be destroyed by the intestinal enzymes. Hence, it is very safe for the consumers. It was also observed that, bacteriocins showed an enhanced zone of inhibition while treating with surfactant *viz.*, Sodium dodecyl sulfate (SDS). But, no clear zone was formed on treatment with urea in comparison to the un-treated control (Fig. 4). It could be assumed that the bacteriocin compound contains a disulphide bond which was cleaved by urea. Since, some of the bacteriocin gene is plasmid associated, an attempt has been made to isolate the plasmid by alkaline lysis method. However, no plasmid could be detected, this would suggest that the Vibriocin isolated in this study is not associated with plasmid.

The cell-free supernatant/crude bacteriocin was further subjected to concentration by lyophilisation technique, and then purified by salt precipitation technique with the use of 60% ammonium sulphate and dialysed with molecular weight cut off of 12000–14000 Da. It has been observed that the antimicrobial activity of the bacteriocin against *S.aureus* and *B. subtilis* was enhanced after concentration and purification.

The purpose of characterisation of bacteriocin was to utilize the antibacterial substance as a bio-preservative in foods. Strong inhibition activity of this potent bacteriocin against *Staphylococcus aureus* could be used as a natural preservative to enhance the shelf life of different processed food products. In future, the conventional and harsh chemical methods can be replaced with a safer and environment friendly bacteriocin.

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