PRODUCTION AND PARTIAL CHARACTERIZATION OF BACTEROCINS LIKE SUBTANCES(BLS) FROM ASSOCIATED BACTERIA ISOLATED FROM MARINE MICROALGAE

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ABSTRACT

In the present study the microalgal associated bacteria were isolated from marine microalgae and screened for the antagonistic property against aquatic pathogens by agar overlay method .The isolates were identified using biochemical tests, and the potent Bacteriocins Like Substances (BLS)producing strain was genetically analyzed through 16S rRNA sequence analysis. The crude bacteriocins substance (BLS)thus obtained was subjected for various physico-chemical characterization. The Zobell's broth and MRS broth have equally supported the bacteriocin production. The maximum yield of BLS was obtained at 48 hrs incubated culture supernatant. The crude BLS showed the best activity in the pH range of 4-8 and it was found to be thermally stable even at 60ºC for 30 minutes and became completely inactive when the temperature reached 110ºC. Addition of 0.5-4% of NaCl had activity and among which the 2.5% maximized the biogenic activity. The activity against the pathogen plated on Muller Hinton Agar increased with decreasing concentration of agar. The molecular weight of the BLS was found to be 12 K Da and 32 K Da. This BLS producing new strain was identified and named as *Vibrio* sp. MMB_2 .As antimicrobial activity against aquatic pathogen, suggested that the present strain can be used as probiotic one in aquaculture industry.

Key words: Agar overlay; algal associated bacteria; Bacteriocins Like Substances (BLS); biogenic activity; *Vibrio* sp. MMB_2;

INTRODUTION

The marine environment harbours a wide range of microbes capable of exhibiting bacteriolytic and antibiotic activity. Marine organisms are a rich source of structurally novel and biologically active metabolites. So far, many chemically unique compounds of marine origin with different biological activity have been isolated and a number of them are under investigation and/or are being developed as new pharmaceuticals (Faulkner, 2000a, b; Schwartsmann *et al,*.2001) Bacteriocins are compounds produced by bacteria that have a biologically active protein moiety and a bactericidal action (Line *et al*., 2008). Bacteriocins are ribosomally synthesized, extracellularly released low molecular-mass peptides or proteins (usually 30–60 amino acids) which have a bactericidal or bacteriostatic effect on other bacteria (Klaenhammer, 1988; Tagg *et al*., 1976), either in the same species (narrow spectrum) or across genera (broad spectrum). Alternate methods for controlling pathogenic bacteriocin including the production of antimicrobial peptides "bacteriocins" are now highly considered. Bacteriocins have extensively been studied with reference to microbiology, biochemistry and molecular biology, because of their applied importance in medicine, pharma-agro and food preservation industries. Small and heat stable peptides containing thio-ether amino acids are classified as Class I bacteriocin (lantibiotics, molecular weight ≤ 5 kDa) Small stable non lantic biotic peptides are class II bacteriocin, molecular weight ≤ 10 kDa (Nes *et al*., 1996). Some large heat stable proteins belong to class III bacteriocins, molecular weight ≥ 10 kDa and class IV are large, complex bacteriocins containing lipid or carbohydrate group (Joerger and Klaenhammer, 1990). Class I and II bacteriocin are the most likely to be used as bio-preservatives in the food industry as a substitute for chemical preservatives among these four groups (Elotmani *et al*., 2002).

The microalgae and algal bacteria are found in symbiotic relationship. This symbiotic bacterium also helps the microalgae to escape from the pathogenic bacteria by competitive exclusion method. The exocellular lytic enzymes are produced by numerous seawater bacteria. The nature of their activity is not specified, they affect most terrestrial germs, which are more sensitive to them than marine bacteria (Jannasch, 1968). These enzymes are most often produced by bacterial symbionts on algae especially by Myxobacteria (cytophaga). It has been evidenced that Bacteriocin Like Inhibitory Substances (BLIS) producing marine bacteria and their use in the control of undesirable bacterial infections with reference to their broad inhibitory spectrum against human, food spoilage and food borne pathogens. Certainly, these BLIS may prove useful alternatives to conventional chemotherapeutics and chemical additives (Zaid Ahmed Pirzado *et al*., 2004). There is growing concern over the development of antibiotic resistance in bacteria. For this reason, the use of probiotic bacteria to prevent or reduce disease is receiving increasing attention as an alternative to antibiotics (Holzapfel and schillinger, 2002; Irianto and Austin, 2002). Therefore, evaluation of probiotic bacteria capable of producing bacteriocin is of intensive research in several sectors in human nutrition, in animal husbandry and fish farming (Hjelm *et al*., 2004). The development of alternative therapeutic agents, less ecologically harmful than usual antibiotics and that do not lead to bacteria resistance is an essential need to a sustainable aquaculture. Hence its hourly needed the eco friendly substance form nature as well as microorganisms. The present study was conducted to isolate the bacteriocin like inhibitary substance producing symbiotic bacterial strain associated with microalgae *Chlorella salina*, and also to isolate and characterize the bacteriocin substance. The activity of the isolated bacteriocin was also tested on the parameters affecting the effect of the bacteriocin when applied in

the field, such as different media pH, temperature, NaCl concentration, time of incubation enzymes, and mode of action of bacteriocin was studied.

MATERIALS AND METHODS

Isolation of marine microalgae associated bacteria

The marine microalgal sample (*Chlorella salina*) was collected from CMFRI at Tuticorin. The collected algal sample was aseptically transferred in a sterilized air tight container. The algae were sub cultured in 500 ml conical flask containing Conway medium. The growth was estimated by cytometric counting. The micro algal associated bacteria were isolated from the growth phase of the algal culture which possessed the highest cell count. From this culture 1ml was taken and serially diluted by serial dilution technique. From the serially diluted samples (10^{-4} to 10^{-6}), 0.1 mL was aseptically transferred and spreaded on Petri plates having Marine Zobell agar. The plates were incubated at 37ºC for 2- 3 days, then the colonies were picked up and transferred to Marine Zobell agar plate for the purpose of pure culture and the isolates were stored in Marine Zobell agar slant for further studies.