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ORIGINAL ARTICLE

ANTIMICROBIAL ACTIVITY OF LECTINS ISOLATED FROM THE HAEMOLYMPH OF MARINE CRAB *Scylla serrata (*FORSKAL – 1775)

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ABSTRACT

Invertebrates need to defend themselves against a variety of pathogens. Lectins are conventionally defined as proteins/glycoproteins of non- immune origin with a remarkable ability to specifically and reversibly interact with carbohydrate ligands. A lectin was purified from the haemolymph of estuarine crab *Scylla serrata*. The investigation was taken against six different species of bacterial strains. The crab haemolymph sample tested against Gram positive *Staphylococcus aureus, Streptococcus pyogenes, and* Gram negative *Escherichia coli, Pseudomonas aeruginosa*, K*lebsiella pneumonia, Bacillus subtilis and Enterococcus faecalis* bacterial strains. The results demonstrated that the antibacterial activity was maximum in *Staphylococcus aureus* and *Pseudomonas aeroginosa* and lowest in *Enterococcus faecalis*. The present study indicates that, the haemolymph of crab would be a good source of antimicrobial peptides.

Keywords: Antimicrobia Activity, Lectins, *Scylla sratta*

1.INTRODUCTION

Some of the invertebrates live in environments rich in potentially harmful microorganisms. They have developed various competent strategies to defend their lives against invading pathogens. Earlier reports suggested that, invertebrates lack the complexity of the adaptive immune system but characterized by some of the known innate immune responses such as phagocytosis, nodulation, encapsulation and synthesis of antimicrobial proteins (AMPs). Antimicrobial proteins are the major components of the innate immune defense system in marine invertebrates. Agglutinins or lectins are conventionally defined as glycoproteins of non- immune origin with a remarkable ability to interact with specific carbohydrate structures present on cell surfaces, extracellular matrices or secreted glycoproteins (Goldstein et al., 1980). They are readily synthesized and efficiently diffuse at the site of pathogen entry or infection (Reddy et al., 2004).

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Scylla serrata is an economically important marine invertebrate distributed throughout the West Pacific and Indian Ocean. It is commonly known as mud crab or mangrove crab. It is the most important edible crab, inhabits muddy bottoms of brackish water along the shoreline, mangrove areas and river mouths. The hemolymph of the marine crab *Scylla serrata* have been studied previously by different investigators. Chattopadhyay and Chatterjee (1997) described the scyllin, an antimicrobial lectins from the hemolymph of *S. serrata*. Hoq and colleagues (2003) identified various proteins fractions from the hemolymph of *S. serrata* and demonstrated antimicrobial activity against various Gram positive and Gram negative bacteria. In the recent past, several pharmacological substances of marine origin have been developed. The chemical structure and biological activity will lead to formulation of new drugs with specific actions. The present investigation was takes up to study the antimicrobial activity of AMP isolated from haemolymph extracted from *S. serrata*.

2.MATERIALS AND METHODS

Collection of experiment animal

Scylla serrata were collected from the sea shore area along the Manakkudi estuary region at Kanyakumari district. They are transported to laboratory with care and kept in cement tank with sea water until further use. Each animal was subjected to a single bleed collection at the time of use. During sample collection time, walking legs of the crab was cut with a fine sterile scissor and approximately 3-4 ml haemolymph was collected in a sterile vessel, which contains sodium citrate buffer, pH 4.6 and equal volume of physiological saline (0.85% NaCl, w/v) which will prevent degranulation and coagulation of haemocytes. Centrifuge the sample at 10,000 rpm at 4^{o}C for 10 minutes. The haemocytes were precipitated and the supernatant was collected by aspirating and stored at 4^{o}C until used. The protein content of the haemolymph and other fractions was estimated by the method of Bradford using BSA as the standard (Brandford, 1976).

SDS-Polyacrylamide Gel Electrophoresis

According to the method of Laemmli, (1970) the agglutinin was performed by electrophoresis in 10% polyacrylamide gel under non denaturing condition at pH 8.9. The band was visualized by 0.2% Coomassie brilliant blue (G 250) staining followed by destaining in 5% acetic acid containing 20% methanol. The sample was treated with 1% SDS in the presence or absence of 2-mercaptoethanol for 5 minutes at 100^{o}C. The molecular mass of the purified *Scylla serrata* lectins (SSL) calculated according to the relative mobility with the Precision plus Protein standard.

Mass spectrometry analysis

The molecular mass was determined by ESI on a Q-ToF2 mass spectrometer (micromass) using sinnapinic acid as the matrix.

Microbial strains and culture

Gram positive bacteria *Staphylococcus aureus,* and *Streptococcus pyogenes,* Gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and K*lebsiella pneumonia, Bacillus subtilis and Enterococcus faecalis* were obtained from the Raja Muthiah Medical College and Hospital, Annamalai University, Annamalainagar.

Antibacterial assay

The spectrum of antimicrobial activity was studied by using the techniques described by Bauer et al., (1996). Take 14ml of bacterial underlay of 1% agarose in 10% MH broth supplemented with 0.02% Tween in a 12 X 12 cm petri dish. The agar was seeded with 1 x 10^{6} washed bacteria. Wells of 3mm diameter were punched into the agarose and 50 µl of the test sample was pipetted into each. Sterile deionized water containing 0.1% acetic acid used as control. The plats were incubated at 4^{o}C for 3 hours and then overlaid with 14 ml of sterile 1% of agarose containing double strength LBB. They were further incubated 24 hours at 39^{o}C. Antimicrobial activity was expressed in terms of diameter of zone of inhibition was measured by using scale and recorded in millimetre.

3.RESULTS

The lectins Scylla serrate antimicrobial protein (SSAP) was purified from the haemolymph of *Scylla serrata* and the electrophoretic analysis analyses using SDS-PAGE and IEF gels were performed. A single protein band corresponding to a molecular mass of approximately 24.0 KDa (fig 1) was observed in SDS-PAGE stained with 0.2% Coomassie brilliant blue (G 250). SSAP was observed as a single peak when applied to an anion exchange

The haemolymph of *Scylla serrata* contains a protein, *Scylla serrata* lectins (SSAP). The antimicrobial activity of the SSAP was tested against six bacterial strains of both Gram positive and Gram negative is summarised in table 1. Among the six strains the maximum diameter of inhibition zone (20 mm) was recorded in *Staphylococcus aureus* and *Pseudomonas aeroginosa* and lowest diameter of inhibition zone (10 mm) was observed in *Enterococcus faecalis.*

Table-1: Antimicrobial activity of the haemolymph of *Scylla serrata.*

S.No	Organisms	Zone of inhibition
1	*Staphylococcus aureus*	20 mm ± 1.17
2	*Pseudomonas aeroginosa*	20 mm ± 1.38
3	*Klebsiella pneumonia*	18 mm ± 1.23
4	*Bacillus subtilis*	16 mm ± 1.13
5	*E. coli*	15 mm ± 1.65
6	*Enterococcus faecalis*	10 mm± 1.43
7	control	0mm

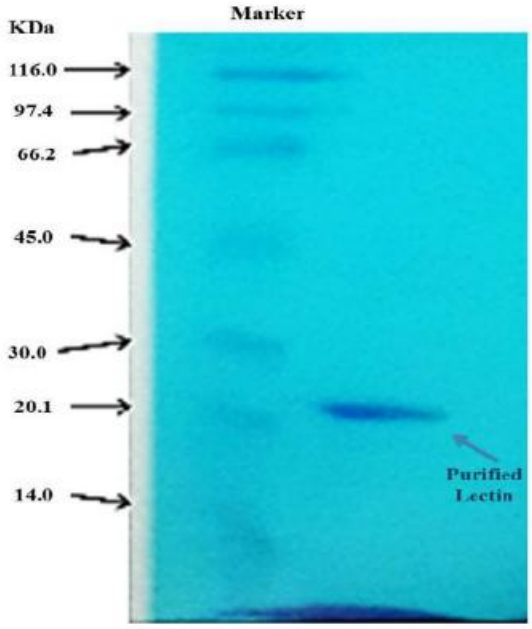

FIG-1 PURIFICATION OF LECTINS FROM SCYLLA SERRATA BY SDS - POLYACRYLAMIDE GEL ELECTROPHORESIS

4.DISCUSSION

Many human pathogens utilize cell surface glycans as either receptors or ligands to initiate adhesion and infection. Cytotoxic effects of lectins may be revealed by deleterious effect on microorganisms. Lectins of different carbohydrate specificities are able to promote growth inhibition or death of bacteria. Antibacterial activity on Gram positive and Gram negative bacterial interaction occurs through the lectins with components of the bacterial cell wall including teichoic and teichuronic acids, peptidoglycans and lipopolysaccharides. *Scylla serrata* is an edible marine crab found in different coastal environment. The environmental factors cause morphological, physiological variations and diversity. Marine invertebrates are rapidly synthesized an antimicrobial peptides (AMPs) as a major component of innate immunity. These molecules have a molecular weight of ≤ 10 KDa, are the first line of host defence in various species. AMPs have microbicide properties against various clinical pathogens including the sexually transmitted infection (STI) causing *Treponema pallium, Chlannydia trachomatis* and HIV (Yedery and Reddy, 2005).

Hog and colleagues (2003) identified various protein fractions from the haemolymph of *S. serrate* that demonstrated antimicrobial activity against various Gram positive and Gram negative bacteria. Recently, Huang et.al., (2006) have isolated a 10.8 KDa anionic protein named scygonadin from the male crab of *S. serrate* that inhibited growth of *micrococcus luteus* and *Aeromonas hydrophila*. Jong Cheon et al., (2011) isolated 24 KDa Philyra Pisum Lectins (PPL) from purse crab *Philyra pisum*, which have mitogenic activity. The present study identification of about 24 KDa antimicrobial proteins from haemolymph of *Scylla serrata* shows high sequences similarity with PPL. The results obtained from the inhibition studies clearly indicate that the serum agglutinins of *S. serrata* interact with a wide range of carbohydrates including acetylated hexosamines, acetylated or non-acetylated sialic acids and several other carbohydrates. The inhibition zones strongly suggest the natural occurrence of multiple agglutinins in the serum of this crab.

In the present study, the extract of *Scylla serrata* haemolymph showed antimicrobial activity against both Gram positive and Gram negative bacteria. A similar result was observed in a haemocytes of Indian mud crab *Scylla serrata* by Roshan Dinesh Yedery et.al, (2009) and a marine crab *O.macrocera* haemolymph by Ravichandran et.al, (2010). The ability of the serum of *S. serrate* to agglutinate the bacteria, particularly the potential pathogens implicates a possible involvement of the humoral agglutinins in host defence response. It is therefore concluded that heamolymph of the marine crab *Scylla serrate* exhibits a wonderful resource of antibacterial proteins, which can be used anti adhesive agents and prevent the colonization of the microbes.

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