

Original Research Article

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Identification and Purification of Antimicrobial Lectins from Marine Crab *Portunus pelagicus* (Linneus, 1775)

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ABSTRACT

Lectins are glycoproteins have the ability to bind carbohydrates. They are involved in biological process such as recognition and binding of carbohydrates, interactions of pathogens, apoptosis, etc. Lectins of different carbohydrate specificities are able to promote growth inhibition or death of bacteria and fungi. In the present investigation made to isolation, identification and antimicrobial activity of lectins from haemolymph of *P. pelagicus*. The haemolymph of *Portunus pelagicus* contains a protein, *Portunus pelagicus* lectins (PPL). The antimicrobial activity of the PPL was tested against six bacterial strains is Gram positive bacteria *Staphylococcus aureus*, and Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Enterococcus faecalis*. Among the six strains the maximum diameter of inhibition zone was recorded in *Staphylococcus aureus* and *Pseudomonas aeruginosa* and lowest diameter of inhibition zone was observed in *Enterococcus faecalis*. It indicates that, the haemolymph of crabs would be a good source of antibacterial agents.

Keywords

Portunus pelagicus
Lectins,
Klebsiella
pneumonia, STI,
AMPs, MIC, LBB.

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Introduction

Lectins are carbohydrate binding proteins have the ability to induce cell agglutination or the precipitation of glyco-conjugates on cell surface receptors. Initially lectins were found and described in plants, later it were isolated from animals and also from microorganisms. However, the yields are usually extremely low. Lectins are glycoprotein have the ability to bind carbohydrates. They are involved in biological process such as recognition and

binding with carbohydrates, interactions with pathogens, cell to cell communication, apoptosis, cancer metastasis and differentiation. The antibacterial, antifungal and antiviral activities of lectins have been reported (Boyel *et al.*, 1997 and Singh *et al.*, 2014). Lectins have become the focus of intense interest for biologists and in particular for the research and application in agriculture and medicine (Movafagh *et al.*, 2013). Lectins

of different carbohydrate specificities are able to promote growth inhibition or death of bacteria and fungi. The inhibition of fungi growth can occur through lectins binding to hyphae resulting poor absorption of nutrients as well as by interference on spore germination process (Lis and Sharon, 1981). Lectins are potential drugs for treatment of AIDS. Lectins (D-mannose-specific) can able to inhibit fusion of HIV infected cells with CD4 cells by a carbohydrate specific interaction with the HIV infected cells (Hansen *et al.*, 1989).

Since ancient time, countries like China, Europe and India, used marine organisms for medicinal purposes. Marine invertebrates are rapidly synthesized an antimicrobial peptides (AMPs). It is a major component of innate immune defence system in marine invertebrates. *Portunus pelagicus* is a medium sized (CL males: 7 cm, females: 6.5 cm) nocturnal marine crab, greenish brown carapace with irregular pale mottling edged dark brown colour. Broad carapace has transverse granulate lines. It is an active swimmer, but during inactive periods buried in sediment. It is the most important edible crab, and a valuable component of small scale coastal fisheries in many countries in tropics (Batooy *et al.*, 1980; Joel and Raj 1987; Mgya *et al.*, 1999). Hudson and Lester (1994) reported that, the crab has close contact with pathogenic bacteria and are prone to infection by microbes at various stages of growth. Cole, (2005) suggested that, AMPs can inhibit the spread of STI and HIV. In the present investigation made to isolation, identification and antimicrobial activity of lectins from haemolymph of *P.pelagicus*.

Materials and Methods

Collection of experiment animal

Portunus pelagicus were collected from the sea shore area along the Managudi 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 cutting with a fine sterile scissor and collect approximately 3-4 ml haemolymph in a sterile vessel, which contains sodium citrate buffer, pH 4.6 and equal volume of physiological saline (0.85% NaCl, w/v). It will prevent degranulation and coagulation of haemocytes. Centrifuge the sample at 10,000 rpm at 4°C for 10 minutes. The haemocytes were precipitated and the supernatant were collected by aspirating and stored at 4°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).

Microbial strains and culture

Gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis* and Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, were obtained from the C.S.I Kalyani Multi Specialty Hospital, Mylopore, Chennai. The organisms were grown in laboratory at 37°C for use for antimicrobial activity.

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 1×10^6 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°C for 3 hours and then overlaid with 14 ml

of sterile 1% of agarose containing double strength LBB (Luria-Bertani Broth). They were further incubated 24 hours at 39°C. Antimicrobial activity was expressed in terms of diameter of zone of inhibition was measured by using scale and recorded in millimetre.

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°C. The molecular mass of the purified *Portunus pelagicus* lectins (PPL) 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.

Results and Discussion

Molecular characterization of *Protunus pelagicus* Lectins (PPL)

The lectins PPL was purified from the haemolymph of *Protunus pelagicus* and the electrophoretic analysis analyses using SDS-PAGE and IEF gels were performed. A single protein band corresponding to a molecular mass of approximately 27.0 KDa (Fig. 1) was observed in SDS-PAGE stained with 0.2% Coomassie brilliant blue (G 250). PPL was observed as a single peak when applied to an anion exchange. The haemolymph of *Protunus pelagicus* contains a protein,

Protunus pelagicus lectins (PPL).The antimicrobial activity of the PPL was tested against six bacterial strains namely, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, are summarized in tables 1 and 2. Among the six strains the maximum diameter of inhibition zone was recorded in *Staphylococcus aureus* and *Pseudomonas aeruginosa* and lowest diameter of inhibition zone was observed in *Enterococcus faecalis*.

Protunus pelagicus 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 pallidum*, *Chlamydia trachomatis* and HIV (Yedety and Reddy, 2005). In the present study, the extract of *P. pelagicus* 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 antimicrobial activity of the PPL was tested against six bacterial strains is summarised in tables. Among the six strains the maximum diameter of inhibition zone (21 mm) was recorded in *Staphylococcus aureus* and *Pseudomonas aeruginosa* and lowest diameter of inhibition zone (10 mm) was observed in *Enterococcus faecalis*. The haemolymphs are the wonderful resource of antibacterial proteins.

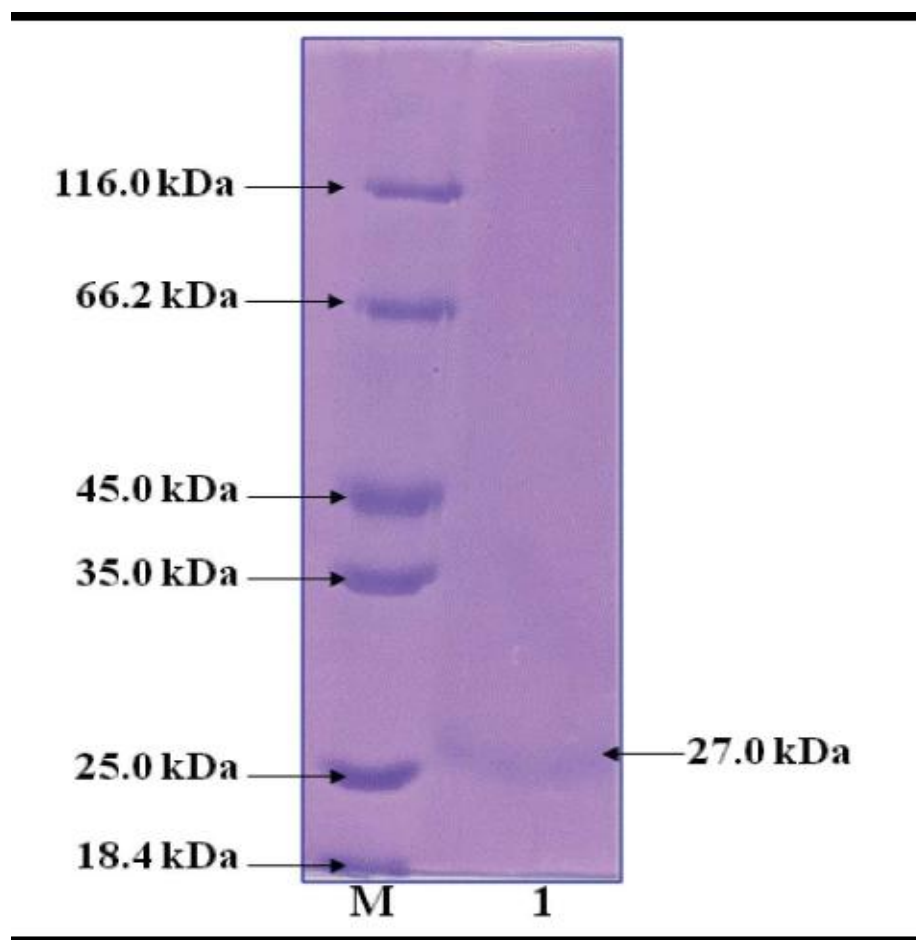
Table.1 Antimicrobial activity of the haemolymph of *Protunus pelagicus*

s.no	Concentration of lectins (µg)	Organisms	Zone of inhibition
1	10	<i>Staphylococcus aureus</i>	7 ± 3.01
2	13	<i>Staphylococcus aureus</i>	11 ± 1.27
3	16	<i>Staphylococcus aureus</i>	15 ± 2.10
4	19	<i>Staphylococcus aureus</i>	18 ± 1.10
5	24	<i>Staphylococcus aureus</i>	21 ± 3.21

Table.2 Antimicrobial activity of the haemolymph of *Protunus pelagicus*

s.no	Concentration of lectins (µg)	Organisms	Zone of inhibition
1	10	<i>Pseudomonas aeruginosa</i>	8 ± 1.02
2	13	<i>Pseudomonas aeruginosa</i>	12 ± 1.15
3	16	<i>Pseudomonas aeruginosa</i>	17 ± 1.25
4	19	<i>Pseudomonas aeruginosa</i>	19 ± 2.30
5	24	<i>Pseudomonas aeruginosa</i>	21 ± 1.15

Fig.1 SDS – gel electrophoresis of purified lectins from *Protunus pelagicus*



The body fluid or hemolymph of almost all invertebrate species contains agglutinins (Yeaton, 1981, Jayaraj *et al.*, 2008). The presence of agglutinins has also been detected in the mucus as well as in certain tissues however its immunological role is best in the hemolymph (Suzuki and Mori., 1991). Recent studies have shown that purified hemolymph served as opsonin in a few insects and mollusks although a number of studies have demonstrated the presence of humoral agglutinins in several crustacean species. It can be noted that the immunological role of these agglutinins remain unknown and that the carbohydrate specificity of serum agglutinins from crustaceans have been elucidated only in a few species (Jayasree, 2001). In the present study describes bacterial growth inhibition activities and carbohydrate specificity of a naturally occurring protein in the serum of the marine crab *Protunus pelagicus*.

In 2010, Petnual *et al.*, found the antimicrobial activity of *Curcuma longa* lectins expressed the minimal inhibitory concentration (MIC) of four microbial species namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* MIC values 0.005, 0.011, 0.002, 0.092 mg/l respectively. From the tested strains *Pseudomonas aeruginosa* shows lowest MIC value 0.002 to be most sensitive to the lectins. In the present study, for 10 µg concentration *Staphylococcus aureus*, shows 7 mm, *Pseudomonas aeruginosa* 8 mm, *Klebsiella pneumonia* 6 mm, *Bacillus subtilis* 7 mm, *Escherichia coli* 6 mm and *Enterococcus faecalis* 4 mm of inhibition zone. From the tested strains *Pseudomonas aeruginosa* shows the maximum zone of inhibition.

Lectins have much attention due to specificity of its interaction with the carbohydrates. The change in cell surface carbohydrates in different pathogenic microbes, lectins as a

therapeutic tool in clinical diagnostics is well established. Many lectins show antibacterial, antiviral and antifungal activities in vitro and optimising their dosage delivery. Thus lectins can be used anti adhesive agents and prevent the colonization of the microbe and establishment of the infection. The present study indicates that the haemolymph of crabs act as a good source of antibacterial peptide agents and would be replace the existing inadequate and cost effective antibiotics.

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