

## Antimicrobial Proteins from the Crab *Charybdis lucifera* (Fabricius, 1798)

G. Rameshkumar, T. Aravindhan and S. Ravichandran

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608 502, India

**Abstract:** The ocean represents a unique resource providing a diverse array of natural products. In the present study antimicrobial proteins was isolated for the first time male and female haemolymph of the crab *Charybdis lucifera*. Crabs were collected from the Vellar estuarine environment. In antibacterial activity the highest zone of inhibition was recorded in male crab (11 mm) against *Escherichia coli* and female crab against *Pseudomonas aeruginosa*. The lowest zone of inhibition was recorded in female crab (7mm) against *Vibrio* sp. But the fungal activity 10 different strains was *Fusarium moniliforme* showing activity (10 mm) rest of them are not showing any activity. After the SDS-PAGE there were two clear bands were detected in the gel that represents proteins of 45 kDa from a female crab *C.lucifera* hemolymph and 25 kDa proteins of male hemolymph crab of *C. lucifera*. In the present study indicated that the haemolymph of *C.lucifera* crabs may potential antibiotics.

**Key words:** Crab · Hemolymph · Antibacterial · Antifungal · SDS-PAGE

### INTRODUCTION

The marine environment has proven to be a source of diverse arrays of bioactive metabolites with great potential for pharmaceutical and other applications [1]. More than 100 pure compounds of known and new structural types have been isolated and characterized. These belongs to different structural types namely 37% of diterpenoids, 18% of steroids/sterol glycosides, 17% of sesquiterpenoids and the remaining were alkaloids, amino acids, fatty acid, alcohol esters glycolipids etc., of the few bio evaluated, some showed interesting biological activity [2]. Humoral immunity in marine invertebrates is characterized by antimicrobial agents present in the blood cells and plasma [3], along with reactions such as hemolymph coagulation or melanization [4,5]. The circulating hemolymph in marine invertebrates contains biologically active substances such as complement, lectins, clotting factors and antimicrobial peptides [6]. Crabs especially mangrove species play an important role in the initial processing of litter in intertidal, riverine and fringing forests [7-12]. Some of the brachyuran crabs have shown profound activity, which is useful in the biomedical area [13,14]. Hence, a broad, based screening of marine crabs for bioactive compounds is necessary. A thorough understanding of

chemical structure and biological activity will lead to the formulation of novel drugs with specific actions. Hence the present study was carried out to antimicrobial protein from the haemolymph of *Charybdis lucifera* crabs in the Vellar estuary.

### MATERIALS AND METHODS

Crabs were collected from the Vellar estuarine environment (Lat 11° 29'N; 79° 46'E) Southeast coast of India. Healthy male and female animals at different stages of development were used throughout for experimental purposes and each animal was subjected to a single bleed collections were being done at the time of use.

**Collection of Haemolymph:** Haemolymph were collected by cutting each walking legs of the animal with a fine sterile scissor. To avoid hemocyte degranulation and coagulation, the haemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, V/V). Equal volume of physiological saline (0.85%, NaCl, w/v) was added to it. To remove hemocytes from the haemolymph was centrifuged at 2000rpm for 15min at 4°C. Supernatant were collected by aspirating and stored at 4°C until use.

**Microbial Strains Used:** Antibacterial activity of crabs haemolymph was determined against 10 bacterial strains viz., *Staphylococcus aureus*, *Salmonella typhi*, *S. paratyphi*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *E.coli*, *Proteus mirabilis*, *Lactobacillus vulgaris*, *Vibrio* sp and *K. pneumonia* and 10 fungal strains *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme*, *Aspergillus terreus*, *Trichoderma* sp. *Penicillium citrinum*, *Aspergillus fumigatus*, *Penicillium oxalicum*, *Rhizopus* sp and *Trichoderma viride*. These pathogens strains were obtained from the Department of medical microbiology (Raja muthiyah medical college and hospital) Annamalai University, Annamalai nagar.

**Anti Microbial Assay:** The spectrum of antibacterial and antifungal activity was studied by using the techniques [15]. Antibacterial and antifungal activity was expressed in terms of diameter of Zone of inhibition was measured in mm using Vernier caliper or a scale and recorded.

**Determination of Molecular Weight by Using SDS-PAGE:** SDS-PAGE is used to find out the molecular weight active fractions of the sample. SDS-PAGE was performed in 12% separating gels, according to the method described by [16].

## RESULTS

**Antimicrobial Assay:** Antibacterial activity of the male and female hemolymph sample of *C.lucifera* was used for the present study. The zone of inhibition in different bacterial strains against *C.lucifera* hemolymph is shown in (Table 1). The measurement of highest zone of inhibition was recorded in male crab (11mm) against *E.coli* and female crab against *P. aeruginosa*. The lowest zone of inhibition was recorded in female crab (7mm) against *Vibrio* sp (Table 1).

Antifungal activity of male and female hemolymph sample of *C.lucifera* was tested against 10 different fungal strains. Among the 10 different strains was *Fusarium moniliforme* showing activity (10 mm) rest of them are not showing any activity (Table 2).

**SDS- PAGE:** The male and female hemolymph samples showing antibacterial activity was subjected to SDS-PAGE to estimate the molecular weight of proteins present in it. Different standard were used to determine the molecular weight of antimicrobial proteins in the hemolymph. The stained gel revealed that the hemolymph

Table 1: Antibacterial activity of hemolymph samples against pathogenic strains (mm)

Pathogenic Microbes	<i>C.lucifera</i> (Male)	<i>C. lucifera</i> (Female)
<i>Staphylococcus aureus</i>	9	10
<i>Salmonella typhi</i>	-	-
<i>Salmonella paratyphi</i>	-	-
<i>Klebsiella oxytoca</i>	10	9
<i>Pseudomonas aeruginosa</i>	10	11
<i>E. coli</i>	11	9
<i>Proteus mirabilis</i>	10	8
<i>Lactobacillus vulgaris</i>	9	9
<i>Vibrio</i> sp.	9	7
<i>Klebsiella pneumonia</i>	9	9

Table 2: AntiFungal activity of animal samples against pathogens Zone of inhibition (mm)

Strains	Zone of Inhibition	
	Malehemolymph	Femalehemolymph
<i>Aspergillus flavus</i>	-	-
<i>Aspergillus niger</i>	-	-
<i>Fusarium moniliforme</i>	-	10
<i>Aspergillus terreus</i>	-	-
<i>Trichoderma</i> sp	-	-
<i>Penicillium citrinum</i>	-	-
<i>Aspergillus fumigatus</i>	-	-
<i>Penicillium oxalicum</i>	-	-
<i>Rhizopus</i> sp	-	-
<i>Trichoderma viride</i>	-	-

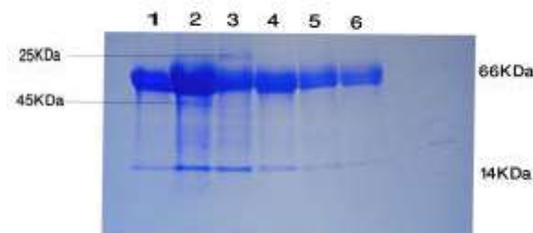


Fig. 1: Molecular weight of hemolymph protein  
25 kDa indicates male crab sample  
45 kDa indicate female crab sample

containing a simple population of proteins. There are different molecular weight marker proteins were used. There were two clear bands were detected in the gel that represents proteins of 45 kDa from a female crab *C.lucifera* hemolymph and 25 kDa proteins of male hemolymph crab of *C. lucifera* (Fig. 1).

## DISCUSSION

Among these 50 have been found to be a widespread use in the prevention and treatment of bacterial disease in animal and man [17]. The first attempt to locate antimicrobial activity in marine organisms was initiated around 1950's [18,19]. A screening of antimicrobial activity of *charybdis* crab species was conducted. In the present study the crab hemolymph shown antimicrobial activity against different range of bacterial strains of both gram positive and gram negative bacteria and pathogenic fungal strains. Previous works shown that marine decapod crustaceans contain factors with antibacterial activity, particularly in the hemolymph or in the hemocytes. This property seems to be a common feature through the order [20]. Antibacterial activity has been previously described in a wide range of crustacean species [21-23].

In arthropods, antimicrobial compounds were mainly studied in chelicerates (Horseshoe crabs) and insects. Their involvement in the defense reaction is quite different in these two groups. The result suggests that brachyuran crabs are not only involves in the economy of finfish resources it can also produce antibacterial substances instantly to combat bacterial infection. Similar result was observed in the hemolymph of some mangrove crabs against clinical pathogens [13]. In the present study indicates that antibacterial activity and the highest zone of inhibition was observed in the hemolymph of *Charybdis* female crabs against *E.coli* sp (11mm) and lowest zone of inhibition in *Charybdis* sample against *vibro* sp of female crab.

The hemocyanins to define variants below the species level; these proteins may exhibit to much adaptive variation, because of their physiological roles, to reflect phylogenetic relationships at the generic level [24]. Molecular weight protein (56.5kDa) was also isolated by [25] in the hemolymph of edible crab *S.serrata* and [26] hemolymph of *Thalamita crenata*. The similar molecular weight proteins isolated from horseshoe crab hemocytes [27]. In *Ceratitis capitata* larvae, a 47 kDa protein on the surface of some hemocytes is involved in the internalization of LPS. The isolation from plasma from the two penaeid shrimp species of novel peptides/ polypeptides with exclusive antifungal activities. A set of three molecules was purified with molecular masses at 2.7 kDa (*Penaeus vannamei*), 7.9 kDa and 8.3 kDa (*Penaeus stylirostris*) [28] The isolation of an approximately 3.7 kDa, basic, antibacterial peptide (designated callinectin), which represents the major

antibiotic activity in blue crab, *Callinectes sapidus*, hemocytes [29]. In the present investigation purified hemolymph sample that showed antibacterial activity was subjected to SDS-PAGE to estimate the number and molecular weight of proteins present. After electrophoresis two clear band were detected in the gel which represents proteins of molecular weight 45kDa and 25kDa.

In the present study indicated that hemolymph of *Charybdis* crabs may contain potential antibiotics. The antimicrobial assays done so far and those that will be done, will serve as a baseline data for further studies that may confirm the hypothesis that brachyuran crabs hemolymph are indeed potential sources of novel compounds with biological potential.

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