Biolife 2(4):1125-1130
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

COMPARATIVE STUDY OF HEMOLYMPH PROTEIN PROFILE IN *BARITELPHUSA CUNICULARIS* AND *PARREYSIA CORRUGATA*

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ABSTRACT

The freshwater molluscs and crustaceans constitute an important component of the ecosystem. The freshwater crab *Baritelphusa cunicularis* and Bivalve *parreysia corrugata* found important component of freshwater ecosystem. Proteins proved versatile macromolecules in living system and served to run biological processes. Immune system of invertebrate must rely on non-self recognized molecules to ensure defense responses against infectious pathogens that continuously threaten their survival. Attempt was made to isolate identify and characterize proteins from crab *Baritelphusa cunicularis* and bivalve *parreysia corrugata* hemolymph using SDS-PAGE. Comparatively higher numbers of proteins bands were found in crab as that of bivalve, which provides differentiating relationship among two phylum's. Protein profiling of crab indicated proteins ranging from 13.39 to 62.73 KDa while in bivalve 17.01 to 61.98 KDa.

Keywords: *Baritelphusa cunicularis*, *Parreysia corrugata*, Hemolymph, Protein profiling, SDS-PAGE.

INTRODUCTION

Proteins content of body is essential constituents in protoplasm and serves for growth and development of organism. Proteins function as catalysts, transport and store other molecules such as oxygen, provide mechanical support and immune protection, generate movement, transmit nerve impulses, and control growth and differentiation (Berg *et.,al* 2002).

Freshwater crabs are an important component of the fauna of limnic environments (Wehrtmann *et al.*, 2010). Crabs are also the best sources for food products including protein source for aquatic lives as well as for human. Crabs, among other invertebrates are considered as an essential shell fishery product (Nalan *et.,al* 2003). About 1300 species of freshwater crabs, distributed throughout the tropics and subtropics regions (Yeo *et al.*, 2008). Freshwater crabs are significant organisms inhabiting Southeast Asian fresh waters, and has key role in recycling nutrients. Recently body metabolites have been investigated as a tool for monitoring physiological condition in wild or cultured crustaceans exposed to varied environmental conditions (Moore *et al.*, 2000; Rosas *et al.*, 2004; Lorenzon *et al.*, 2007). Blood of crab has two components, hemolymph plasma and hemocytes, which play major roles in their immune mechanism. The nutritional quality of the crab proteins is very favorable when compared with other poultry animals. There are conflicting reports on the effects of nutritional status upon blood protein concentrations in haemocyanin-containing species.

The haemolymph proteins of invertebrates are unique in composition, as they do not contain immunoglobulin or albumin like proteins and the protein composition varied in relation to physiological and functional state of the animal. The relative contributions of haemocyte phenoloxidase and hemocyanin in the standard physiological ratio at which they occur in haemolymph was recorded in the crab, *Cancer magister* (Terwilliger *et.,al* 1968). The concentration of protein in the haemolymph showed wide difference among brachyuran crabs like *Carcinus maenas* and *Uca minax* (Depeledge *et.,al* 1989) in which, 70-95% consists of the respiratory pigment haemocyanin. The blood constituents vary depending on physiological state and hence found potential indicators in animal (Sastry and Miller, 1981).

The freshwater bivalve *Parreysia corrugata* found widely distributed and reported to be medicinally important (Dey, 2008) and used by indigenous people to control blood pressure (Prabhakar and Roy, 2009). It is also used in cement, lime, button, toys and cosmetic industries. In certain parts of the country, the animal is consumed as food by economic people. Recently pearl production has been reported using these animals in the state of Orissa (Janakiram and Misra, 2003).

In this present study, electrophoretic analysis of hemolymph proteins of freshwater crab *Baritelphusa cunicularis* and bivalve *Parreysia corrugata* was analyzed in relation to its quantitive and qualitative difference of hemolymph protein concentration, applying SDS-PAGE. Obtained protein profiling among the two groups was comparatively interpreted for to get the differentiation among the invertebrate fauna and their sustainable development against contaminated aquatic media.

MATERIALS AND METHODS

Electrophoresis is stabilizing media has been widely used for accurate and rapid characterization of haemolymph proteins. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a common technique for separating proteins at good resolution.

Study area and Animal collection:

The fresh water crab *Baritelphusa cunicularis* and bivalve *Parreysia corrugata* were collected at the morning in between 8:00 am to 10:00 am. in summer season from the Hiranyakeshi river,(Mahagoan spot) from Gadhinglaj tahsil Dist. Kolhapur (M.S). After collection, the crab and bivalves were brought to the laboratory. Healthy crabs having equal size (carapace width 13.1- 13.5 cm and body weight 215-217 gm) were used for experiment. The hemolymph was collected directly from adductor muscles.

Collection of Haemolymph:

In crab hemolymph was obtained from the ventral part of the abdominal segment using a fine sterile 25 gauge needle and a 1ml syringe, while in bivalve the hemolymph collected from adductor muscles. To remove haemocytes from the hemolymph it was centrifuged at 3000 rpm for 10min. Supernatant was collected for the biochemical analysis by following standard procedure i.e. Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Molecular weight determination by SDS PAGE:

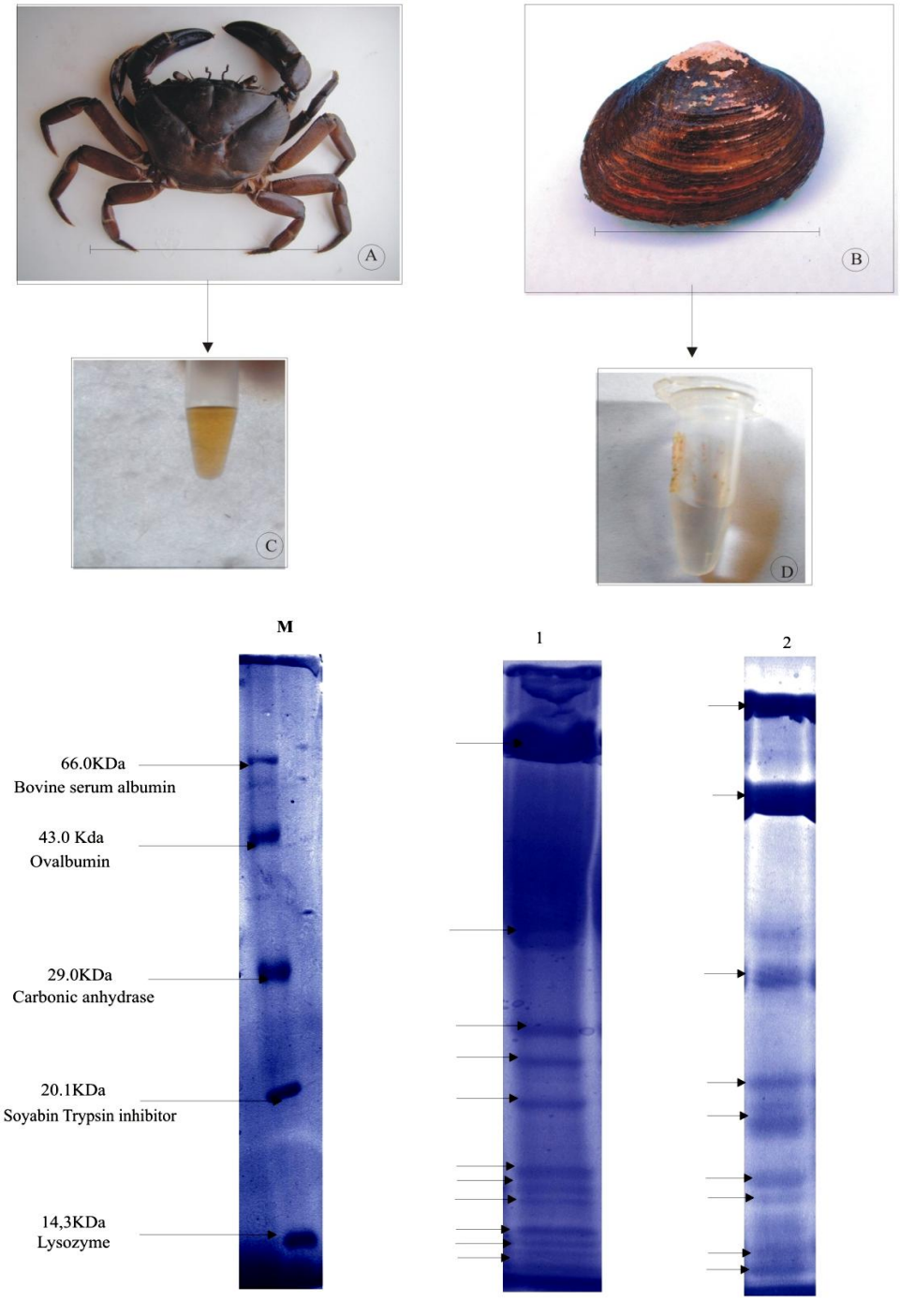
SDS PAGE was performed according to the method of (Laemmli, 1970) using a slab gel with a linear gradient of 4-12% gel. The gel was run at a constant voltage of 250V for 6 hours. The molecular mass of the subunit of normal and challenged haemolymph was estimated by measuring its relative mobility in SDS-PAGE compared to those of the low molecular weight standards. A mixture of crab and bivalve proteins, from 14.3 to 66.0KDa, was used as protein markers. Protein bands were stained with Coomasie Blue (0.02% Coomassie brilliant blue R-250 in 50% methanol -7.5% of acetic acid). The haemolymph of the animal which showed maximum protein profile was subjected to SDS PAGE. The comparative account of molecular weight of the protein constituents of haemolymph of crab and bivalve was analyzed with standard protein marker.

RESULTS

In the present study the haemolymph of crab and bivalve were used to estimate the molecular weight of proteins present in it by SDS – PAGE. The proteins were separated by SDS-PAGE and size of polypeptide chains of the given crude hemolymph protein were determined by comparing its electrophoretic mobility in SDS-PAGE gel with mobility of marker proteins of known molecular weight. SDS-PAGE was performed in 12% separating gels, 4% stacking gel according to the method described by Lammeli *et al.*, (1970).

The term protein profiling refers to the

Fig: 1 A: Freshwater crab, B: Freshwater bivalve, C and D: Hemolymph of respective animals, M: Standard protein marker, Lane 1: Protein separation in Freshwater crab, Lane 2: Protein separation in Freshwater Bivalve.



extraction, separation and characterization of proteins expressed in an individual. Protein concentration of crab and bivalve were well reflected in electropherogram (Fig.1). As per molecular weight marker proteins, the maximum protein profile was found in the hemolymph of crab *Baritelphusa cunicularis* (Fig. 1 lane 1) while comparatively less protein profile was observed in bivalve *Parreysia corrugata* (Fig. 1 lane 2). In crab *Baritelphusa cunicularis* haemolymph, the proteins ranging from 14.3 to 66.0 KDa. Eleven clear bands were observed in *Baritelphusa cunicularis* (Fig.1 lane1). While in bivalve haemolymph of *parreysia corrugata* nine clear protein bands were observed and were ranged from 14.3 to 66.0 KDa. (Fig. 1 lane 2). The staining gel revealed clear 43KDa (Ovalbumin) band. Very thin and clear bands were observed in between lysozyme to soyabean trypsin inhibitor (14.3 to 20.1KDa).

The hemolymph concentration was found to be higher in crab as that of bivalve (Fig. 1). Most of the proteins were compiled in between carbonic unhydrase and ovalbumin (29.0 to 43.0 KDa) (Fig. 1 lane 1). In case of *Parreysia corrugata* very thick fine band was compiled in between bovine serum albumin and ovalbumin (43.0 to 66.0 KDa) (Fig. 1 lane 2). Carbonic unhydrase with molecular weight 29.0KDa was clearly observed. Two fine clear bands were observed near soybean trypsin inhibitor protein with molecular weight 20.1 KDa. Thus the protein bands observed in hemolymph of crab and bivalve are listed along with their molecular weights. Hemolymph concentration listed with their staining intensity (Table No. 1).

Table No. 1: Details of proteins isolated and characterized from crab and bivalve hemolymph

Animals	Protein bands with molecular weight(KDa)
Baritelphusa cunicularis	13.39, 24.02, 33.55, 34.97, 36.46, 44.92, 46.84, 48.83, 55.37, 57.58, 62.73.
Parreysia corrugata	17.01, 23.51, 27.56, 37.76, 40.71, 47.35, 54.46, 58.72, 61.98

DISCUSSION

The hemolymph of decapods has received considerable attention by a number of crustacean physiologists because of the wide range of variability observed in its constituents. Crustacean haemolymph proteins vary with nutritional state. Blood proteins considerably changed during imposed fasting, qualitative studies of blood protein were mainly based on electrophoretic mobility of proteins. (Florkin, 1960; Engle and Woods, 1960; Gibert, 1972). Thus, in the present study the protein variations observed in bivalve and crab might be due to its varied nutritional state. Factors like sex, size, moult cycle and environment may play a vital role in the variation of hemolymph proteins. The biochemical constituents of the animal are known to vary with season, size of the animal, stage of maturity, temperature and available of food etc. (Akabar 1988, Soundarapandian 2008).

Protein found essential for the sustenance of the life and growth and hence has major concentration in body. Proteins proved prominent biochemical component of crustaceans from eggs to adults and is strikingly dominant in younger phases. The protein content in the crab was reported to be higher in hard shell crabs (Sudhakar *et al.*, 2009). In the present study the protein content in circulating haemolymph was more in crab which might be due to its transportation protein for new cuticle formation. Haemolymph of the brachyuran crustacean *Callinectes sapidus* possess bactericidal activity against *A. hydrophila*, *V.parahemolyticus*, *V. alginolyticus* and *V. ulnificus* (Edward *et al.*, 1996). Antibacterial activity has been reported in the haemolymph of the blue crab *Callinectes sapidus*, mud crab *Scylla serrata* and *Ocypode macrocera* (Hoq *et al.*, 2003; Ravichandran *et al.*, 2010). In the present study *Baritelphusa cunicularis* showed many protein bands with molecular size ranging from 22 to 60KDa. (Lane.1) Similarly SDS-PAGE of haemocyte contents showed protein bands with molecular size ranging from 22 to 91KDa was reported by Samuthirapandian *et al.*, (2010). Likewise Pan *et al.*, (2008), reported two bands at molecular weight around 73 and 75

KDa from haemocyte samples of *Litopenaeus vannamei.*

Thus assessment of hemolymph proteins in crab and bivalve species revealed a wide range of variation in protein profile. The electrophoretic pattern of hemolymph proteins of *P. hydrodromous* reveals three distinct zones, which are evident in the running gel. The proximal zone of the electropherogram corresponds to the upper region of the classification of zones of Maguire and Fielder, (1975) where they found three fractions in *Scylla serrata.* In the region of the electropherograms of hemolymph of *S. serrata,* Kannupandi and Paulpandian, (1975) have reported 8 fractions. In addition to hemocyanin, other proteins like glycol or lipoproteins, globulins, coagulin may also be present in the hemolymph of crustaceans. Chan *et al.*, (1988) have reported that hemocyanin is the major protein detected in different species which accounts for 80 to 95% of the total hemolymph protein. The rest of the protein may consist of mostly free enzymes (Scheer, 1960).

CONCLUSION

The present study enlights informative and useful data about hemolymph content from species of crustaceans and molluscs. Study attended basic diversified protein profiling among the two species which will be useful in the monitoring of the biochemical or physiological state of the animal under various conditions.

ACKNOWLEDGEMENT

Authors are thankful to the Head, Department of Zoology, Shivaji University Kolhapur and Dr. Ghali College Gadhinglaj for providing facilities in the progress of work.

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