Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (2):** 456-461 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Research Article

OPEN ACCESS

Status of Electrolytes and Trace Elements in the Tissue of *Macrobrachium rosenbergii* (De Man) Inoculated by *Pseudomonas aeruginosa* MTCC 1688

K. Ramalingam[1&2]*, S. Ramarani[1], R. Karthik[2] and R. Muthezhilan[2]

[1]PG (Aquaculture) and Research Department of Advanced Zoology and Biotechnology,
Government Arts College, Nandanam, Chennai-600 035, India

[2]Department of Marine Biotechnology & Centre for Marine Bioprospecting
AMET Deemed to be University (U/S 3 of UGC Act 1956), Kanathur, Chennai 603112, Tamil Nadu, India

*Corresponding Author E-mail: krmbiomed@gmail.com

ABSTRACT

The giant freshwater prawn Macrobrachium rosenbergii was injected with an inoculum containing LD50 96 hr dose of 10^7 Pseudomonas aeruginosa (MTCC 1688) to determine the electrolytes and trace elements effects in vivo. There was a significant increase in sodium content in the body muscle and hepatopancreas and a significant decrease in the haemolymph after 96 hours of inoculation of Macrobrachium rosenbergii with Pseudomonas aeruginosa MTCC 1688. Potassium Decrease in potassium content in the body muscle and hepatopancreas, where as a significant increase in the haemolymph of were observed in Macrobrachium rosenbergii inoculated with Pseudomonas aeruginosa in contrast to the sodium content referred to above. The present study also brings about the negative correlation with reference to the sodium and potassium concentrations in the haemolymph. Among the trace elements, copper content resulted in a significant decrease in the body muscle and insignificant decrease in the chitin of Macrobrachium rosenbergii inoculated with Pseudomonas aeruginosa. Considering the above results on copper, lead, iron and zinc the conspicuous decrease of lead and iron in all tissues, while decrease of copper in muscle and chitin while increase of it and zinc in hepatopancreas reveal the differential values of the above trace elements in Macrobrachium rosenbergii. As these elements are essential prerequisites for shrimps in their moult cycle, their reduction in their tissue reservoirs of inoculums treated forms are of significance in aquaculture.

***Keywords**: Pseudomonas, electrolytes, trace minerals, Macrobrachium rosenbergii, aquaculture.*

INTRODUCTION

The rapid growth of human population particularly in the developing countries and the consequence increase in food requirement exerts pressure to exploit the available resources of nature. Wild water bodies represent one of the natural resources for human food. The increasingly limited opportunities in the capture fisheries sector have generated considerable interest in artificial aquaculture. Among that, shrimp farming is one of the most important aquaculture in worldwide especially in Asia due to their economic value. Recently, it is estimated that approximately more than 5 million metric tons of shrimp are annually produced but the current global demand for both the wild and farmed shrimp is approximately more than 6.5 million metric tons per annum[11]. So, in recent times there are many shrimp farms are being created throughout the world to solve this increasing food demands[7]. However, fast development of these shrimp industry has produced various ecological, economical and social issues.

The culture practices of freshwater prawn, particularly, the giant river prawn, *Macrobrachium rosenbergii* known as scampi is rapidly expanding in many countries. In India, *M. rosenbergii* is one of the important commercial crustaceans as it has good demand in domestic and export markets[6,16]. Production of healthy and quality seeds have been a major obstacle in expansion of the culture of *M. rosenbergii*. A complex of environmental factors, microbiological profiles and management practices influence the success of the production cycle. In rearing *M. rosenbergii*, survival rates are dependent on several factors such as stocking densities,[15] water volume or surface area[5], pH level of water and food supplement[1].

In general disease outbreaks in grow out ponds have been a perennial threat to their growth and productivity. Such diseases also bring heavy economic loss for the culturists and investors due to mass mortality. Both bacterial and viral diseases have been established in prawn/shrimp culture. [18] The literature has revealed that both pathogenic and non pathogenic bacteria population exists in natural freshwater and marine water sources. The complex nature of these disease outbreaks in crustaceans has necessitated the need for an understanding of the causative pathogens and their metabolism and the progress of their infection in vivo in order to apply control measures and managerial strategies. The gross and histopathological findings, both at macro (gross) and micro level, are of diagnostic value in the identification of the nature of infection. Such identification/prognostic analysis of symptoms in shrimp culture farms would go a long way in minimizing the risks involved or in eliminating the pathogenic population from water[20].

Towards this end, a time bound analysis of tissues of prawns, after the inoculation of the pathogen would be of value in assessing the pathological effects over a specific period. Since mortalities have been reported to begin towards the end of the grow out period when the shrimps approach the market size, the diagnosis of pathogenic infection is crucial and prudent at this stage. In the present study, the giant freshwater prawn inoculated with an LD50 96 hr dose of *Pseudomonas aeruginosa* MTCC 1688 was subjected to electrolytes and trace elements analysis.

MATERIALS AND METHODS

Specimen collection

Specimens of *Macrobrachium rosenbergii* were collected from the commercial shrimp farm at Kanathur, along the coastal area of Chennai. After being brought to the laboratory, the giant prawns were acclimated to the laboratory conditions in the stocking glass tanks (salinity 2 ppt, temperature 28 + 2^{o}C). The prawns were kept for a minimum of 15 days prior to the experimentation as suggested by Drach for aquatic crustaceans[4].

Procurement and culture of *Pseudomonas aeruginosa* MTCC 1688

The bacterial strain *Pseudomonas aeruginosa* MTCC 1688 used in this study was brought from the Institute of Microbial Type Culture Collection and Gene Bank, Chandigarh, India. Culture was done as prescribed by the above Institute.

Preparation and inoculation of *Pseudomonas aeruginosa* MTCC 1688

The bacterial inoculum was prepared by the procedure adopted by Lightner and Lewis[13]. The live bacteria were harvested from 24 hr culture using sterile bacterial loop and mixed with double distilled water. This was then diluted to two-fold serial dilutions of the bacterial suspension, which was made into different dilutions viz., 10^8, 10^7, 10^6, and 10^5. About 0.05 ml of the inoculums of the different dilutions was taken in 1 ml tuberculin syringe and injected in between 5th and 6th abdominal segment of *Macrobrachium rosenbergii*. The LD50 concentration of inoculums was 10^7. The bacterial count for LD50 value of inoculums was calculated as 2.41 x 10^7 CFU/0.05 ml. The above LD50 dose of 107 was taken for the study and injected into the prawns.

Determination of water content

The water content in the haemolymph and tissues (body muscles and hepatopancreas) were determined by gravimetric method.[19] The haemolymph sample (0.1ml) was collected in a previously weighed dry cover slip and dried at 100^{0}Cin an oven for 16 hours. The cover slip with dried sample was weighed again to determine the water content.

From hepatopancreas and muscles, samples of 100mg were taken for the above study. Six samples for each tissue were taken for analysis.

Electrolytes

The electrolytes like sodium and potassium were estimated by flame photometry method (Systronics Flame photometer, 121). The procedure of tissue extraction was followed by the procedure of Bellamy and Jones[3]. The tissues of the muscle and hepatopancreas were rapidly dissected out and weighed. Pooled tissue of 250 mg from each organ was placed in small beaker with 25 ml of analar nitric acid and the contents were heated to 60^0C to digest the tissues. The nitric acid was diluted appropriately for the estimation of sodium and potassium.

Atomic Absorption Spectrophotometry

Determination of Trace elements (Cu, Fe, Pb, Zn)

The atomic absorption spectrophotometer Perkin-Elmer model 2380 was utilized for analyzing all the trace elements (Cu, Fe, Pb & Zn. Radio-Isotope Department, Veterinary college, Chennai). 5 gm of accurately weighed tissue was taken in a 125 ml Erlenmeyer flask and added glass beads and 25 ml of deionized water. 10 ml of 1+1 mixture of conc. Nitric acid and Perchloric acid were added. The sample was boiled to get a clear solution. The solution was transferred quantitatively to a 100 ml of volumetric flask and diluted with deionized water and mixed thoroughly. About 2 ml of solution was utilized for reach measurement. According to the absorbance, the concentration was calibrated and the concentration was measured directly when the sample was within the linear working range.

RESULTS AND DISCUSSION

The increased water content in all the three tissues of the *Pseudomonas aeruginosa* inoculated *Macrobrachium rosenbergii* is attributed to the proteolytic activity in vivo in the host tissues. As metabolites inside the tissues are being mobilized by the extracellular enzymes, proteases, peptidases and lipases by bacterial colony in vivo for its growth and metabolism, the increase in percent water content may not be unexpected. In this context, Shyamala[21] has reported the depletion of host tissue free sugars like pentose, hexose and ketose, proteins as well as the total lipids in *Penaeus monodon* inoculated with *Vibrio parahaemolyticus* MTCC 451. She has also noticed a significant increase in percent water content in the three tissues body muscle, hepatopancreas and haemolymph. Hence the dilution effect of metabolites withdrawal may be attributed for the increase in percent water content.

Electrolytes: Sodium

Freshwater prawns are less stressed by changes in the ambient conditions with regard to their marine counterparts. As the water and electrolyte status may serve as a sensitive physiological index, the ionic changes occurring in the tissues of the freshwater prawn, *Macrobrachium rosenbergii* were analyzed. In the present study, there was a significant increase in sodium content in the body muscle and hepatopancreas and a significant decrease in the haemolymph after 96 hours of inoculation of *Macrobrachium rosenbergii* with *Pseudomonas aeruginosa* MTCC 1688.

Similar increase in sodium content in the tissues of animals has been revealed in toxicological studies. Tulasi *et al* [22] reported an increase in sodium content of the fresh water crab Barytelphusa guerini treated with sub lethal concentrations of lead acetate and lead nitrate. Vijayaraman[23] revealed an increase in sodium content in the tissues of freshwater prawn *Macrobrachium malcolmsonii* exposed to heavy metals such as cadmium, copper, chromium and zinc. In the present study, the increase in the tissue sodium levels as well as the marked decrease in the haemolymph both could be attributed to the microbial metabolism and its action. The increase in the tissue sodium may be possible due to permeability changes of the cell membrane and their consequent uptake from the extracellular environments. On the country, the decrease in the sodium content of the blood may be attributed to the growth and multiplication of the bacteria, which need the above cationic element for their growth[12].

Potassium

Potassium plays an important role in the maintenance of the electrochemical balance of the tissues. This ion is an important constituent of the extracellular fluid with a marked influence on muscle activity.

Moreover potassium is the principal cations of the intracellular spaces and plays a vital role in various physiological functions involving nerve and muscle acid-base balance and osmotic pressure. In the present investigation a decrease in potassium content in the body muscle and haepatopancreas, where as a significant increase in the haemolymph of were observed in *Macrobrachium rosenbergii* inoculated with *Pseudomonas aeruginosa* in contrast to the sodium content referred to above. The increase in potassium in haemolymph, while its decrease in tissues indicates the disruption of the ionic equilibrium in the tissues of the infected prawns. Similar changes with regard to sodium and potassium have also been recently observed by Shyamala[21] in *Penaeus monodon* infected with *Vibrio parahaemolyticus* MTCC451.

The present study also brings about the negative correlation with reference to the sodium and potassium concentrations in the haemolymph. There is no direct evidence to relate tissue degenerative changes to the electrolyte concentrations especially the cationic group of elements. Experimental evidences in animals have delineated the chemistry of necrosis and revealed that the degenerative changes are due to collagenolytic activity and the attendant changes involving substances like carbohydrates, proteins and hexose amines etc. These studies have also revealed the loss of cationic elements due to general proteolytic degradation. The changes in the sodium and potassium in the infected prawns may be taken as a sequel event consequent to proteolysis to cause inflammatory changes and tissue damage. The endotoxin stress might have caused impairmrnt in the tissue level electrolytes of cationic nature.

Trace Elements (Cu.Pb,Fe and Zn)

In the present study, the copper content resulted in a significant decrease in the body muscle and insignificant decrease in the chitin of *Macrobrachium rosenbergii* inoculated with *Pseudomonas aeruginosa*. In contrast, the copper content in the hepatopancreas showed an insignificant increase, while its concentration in the haemolymph showed a significant increase.

The atomic copper being the prosthetic component of the respiratory pigment haemocyanin in these crustacean species; its increase in the haemolymph may not be unexpected. However, its decrease in tissues, muscles and chitin, which represent sequestering reserve tissues for copper content, is of interest in infection.

Similarly, White and Rainbow[25] observed the uneven distribution of copper in different tissues of *Palaemon elegans* and observed highest concentrations of copper binding components in hepatopancreas. Vogt and Quintio[24] observed the accumulation of high quantity of copper granules in the hepatopancreatic tubules. The decrease in the total haemocytes count in the present study reveals the inhibition in the synthesis of haemocyanin for the formation of haemocytes and thereby resulted in the increased level of copper content in the hepatopancreas and hemolymph.

In the present study, the lead content resulted in a significant decrease in all the tissues, viz., body muscle, hepatopancreas, haemolymph and chitin of *Macrobrachium rosenbergii* inoculated with *Pseudomonas aeruginosa*. Ghidalia *et al.*[9] reported the binding of iron to haemocyanin in decapods. It is reported that iron is stored in granular form in the hepatopancreas in the decapods[17]. In the present study, the zinc content resulted in an increase in the tissues viz., body muscle, hepatopancreas and chitin expect the haemolymph where it decreased. The distribution of zinc content in different tissues of decapods crustaceans revealed that certain tissues like hepatopancreas and gills may be involved in the process of uptake and its storage[2,14,26]. Some studies revealed the binding of heavy metals to the proteins of the haemolymph[8,27] It has also been suggested that the haemocyanins of crustacean facilitate the uptake of zinc via the zinc-haemocynanin complex[2,10].

REFERENCES

1. Barros, H.P. and Valenti, W.C. Food intake of *Macrobrachium rosenbergii* during larval development. *Aquaculture*, **216**: 165 176 (2003)
2. Bryan, G.W. Zinc regulation in the lobster Homorus vulgaris. I.Tissue zinc and copper concentrations. *J. mar. boil.* U.K., **44**:549-563 (1964)
3. Bellamy, D. and Jones, I.C. Studies on Myxine glutinosa - I. The chemical composition of the tissues. *Comp. Biochem. Physiol.*, **3**: 175-183 (1961)

4. Drach, P. Mue et cycle d' intermue chez les crustaces Decapodes. *Ann. Inst. Ocean*. Paris, **19**: 103-391 (1939)
5. D Abramo, L.R. Daniels, W.H. Gerard, P.D. Jun, W.H. and Summerlin, C.G. Influence of water volume, surface area, and water replacement rate on weight gain of juvenile freshwater prawns, *Macrobrachium rosenbergii*. *Aquaculture*, **182**: 161 171 (2000)
6. FAO, National Aquaculture Sector Overview, India. Food and Agriculture, Organization of the United Nations (2013)
7. Food and Agriculture Organization, The state of world fisheries and aquaculture. Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy (2012)
8. Guary, J.C and Nagrel, R. Plutonium and iron association with metal binding proteins in the crab (Cabcer pagurus(L). *J. exp. mar. Boil, Ecol.*, **42**: 87-98 (1980)
9. Ghidalia, W. Fine, J.M. and Murex, M. On the presence of an iron binding protein in the serum of a decapods crustacean (Maropipus puber(Linne)). *Comp. Biochem. Physiol.*, **41 B**:349-54 (1972)
10. Jhonson, I. The effects of combination of heavy metals, hypoxia and salinity on the Iron regulation in Cragon crangon(L.). and Carcinus amenus(L.). *Comp. Biochem. Physiol.*, **11(3)**: 227-257 (1988)
11. Karthik, R. Jaffar Hussain, A. and Muthezhilan, R. Effectiveness of *Lactobacillus* sp (AMET1506) as Probiotic against Vibriosis in *Penaeus monodon* and *Litopenaeus vannamei* Shrimp Aquaculture. *Biosciences Biotechnology Research Asia*, **11**: 297-305 (2014)
12. Lighter, D.V. Vibriosis culture and Identification. In: A handbook of pathology and diagnostic procedures for disease of Penaid shrimp. The world Aquaculture Society. Louisiana State University, Baton Rouge, Louisiana. 1-27 (1996)
13. Lightner, D.V. and Lewis, D.H. A septicemic bacterial disease syndrome of penaeid shrimp. *Mar. Fish. Rev.*, **37(5-6)**: 25-28 (1975)
14. Martin, J.L.M. Van, W.A and Ceccadi, H.J. Zinc hemocyanin binding in the hemolymp of Carcinus maenus (crustacean, decapoda). *Comp. Biochem. Physiol.*, **58A**: 193-195 (1977)
15. Marques, H.L.A. Lombardi, J.V. Boock, M.V. Stocking densities for nursery phase culture of freshwater prawn *Macrobrachium rosenbergii* in cages. *Aquaculture*, **187**: 127 132 (2000)
16. MPEDA, Statistics of Marine Products Exports 2010. The Marine Products Exports Development Authority, Kochi. pp. 360 (2010)
17. Ogura, K. MIidgut gland cells accumulating iron or copper in the crayfish, Procambarus clarkia. *Annot. Zool. Jap.*, **32**: 133-142 (1959)
18. Ramalingam, K. and Ramarani, S. Pathogenic changes due to inoculation of gram-negative bacteria Pseudomonas aeruginosa (MTCC 1688) on host tissue proteins and enzymes of the freshwater prawn, Macrobrachium rosenbergii (De man). *J. Environ. Biol.*, **27**: 199-205 (2006)
19. Ramalingam, K. Ravindranath, M.H. Ramalingam, K. Effects of autonomy and bleeding stress on the hemolymph protein concentration and soluble nitrogen of the shore crab Ocypode platytarsis. *Crustaceana*, **40**: 88 (1980)
20. Ramalingam, K. and Ramarani, S. Effect of Pseudomonas aeruginosa on the giant freshwater prawn, Macrobrachium rosenbergii - histopathological and electron microscopic study. *Journal of Environmental Biology.* **28(3)**:627-635 (2007)
21. Shymala, D.R. Studies on the infection of an endemic species, *Vibrio parahaemolyticus* MTCC 451 and its manifestations on blood and tissue metabolic profiles of the black tiger prawn, penaeus monodon (Fabticius). Ph.D.Thesis, University of Madras (2001)
22. Tulasi, S.J. Yasmeen, R. and Rao, R. Ionic balance in the haemolymph of freshwater crab, Barytelpusa guerini. .*J.Envir.Biol.*, **11**: 163-168 (1990)
23. Vijaraman, K. Physiological responses of the fresh water prawn *Macobrachium malcolmsoni* (H. Milne Edwards) to the heavy metals cadmium, copper, chromium and Zinc. Ph.D. Thesis. Bharathithasan University, India (1993)
24. Vogt, G. and Quinito, E.T. Fate and effects of after borne heavy metals in penaeus monodon. *Aquacult. Environm.*, **14**: 320-321 (1991)

25. White, S.L. and Rainbow, P.S. On the metabolic requirements for copper and zinc in mollusks and crustaceans. *Mar. Envir .Res.*, **16**:215-229 (1985)
26. White, S.L. and Rainbow, P.S. Regulation and accumulation of copper, zinc and cadmium by the shrimp Palaemon elegans. *Mar. Ecol. Prog.Ser.*, **8**: 95-101 (1982)
27. Zatta, J.E. In ; Biostatical Analysis Prentice Hall Inc., U.S.A (1974)