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Comparative Account of DNA Extraction Protocols in Some Freshwater Prawns of Genus *Macrobrachium* (Bate, 1868) (Family Palaemonidae) from Jammu Waters for PCR Based Applications

Raman Jasrotia* and Seema Langer

Animal Cytogenetics Lab, Department of Zoology, University of Jammu, Jammu (180006), India.
*Corresponding author email: ramanjasrotia18@gmail.com

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Genetic variations among prawns act as an important tool to characterize and differentiate between the species. Molecular and phylogenetic analysis of shrimps and prawns like any other organism rely on high yields of pure and better quality genomic DNA. In this regard isolation of DNA is the first and basic step. In spite of the availability of many protocols of DNA extraction from animal tissues, it is difficult to ascertain that which one would provide desired results for prawn tissue. In the present study, three different techniques of DNA isolation i.e., salting out, phenol-chloroform and Qiagen DNA extraction kit were performed and compared for their yield. Cephalothoracic tissue and muscle tissue of pleopods were used for isolation. Tissue samples from fresh specimens as well as from alcohol preserved specimens were employed for extraction. The quantity (µg/ml) and quality of isolated DNA were determined by UV spectrophotometry and agarose gel electrophoresis. Results showed that Phenol-chloroform method with slight modifications obtained higher yield of genomic DNA as compared to other methods. The present work also revealed that among fresh specimens cephalotoracic tissue yielded high concentration DNA than muscle tissue. However, among alcohol preserved specimens, the concentration of DNA was higher in muscle tissue of pleopods. The high quality DNA was then subjected to randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) analysis. The DNAs produced clear, sharp and reproducible PCR (Polymerse chain reaction) product pattern.

Keywords: DNA extraction, Genetic variations, ISSR, PCR, Phenol-chloroform, RAPD, Spectrophotometry.

Aquaculture has become an emerging field to meet the nutritional and economic needs of man in 21st century. In this context, culture of fishes and shell fishes (prawns, shrimps and crabs) on a large scale is contributing significantly to achieve global food security targets. The fresh water prawns besides their high dietary value have significant medical importance too as some of the *Macrobrachium* species serve an imperative role in the biological control of human schistosomiasis by acting as predators of the snail species which are the intermediate hosts of the parasite *Schistosoma*[1,2].

In Jammu division of J&K state, several prawn species such as *Macrobrachium dayanum*,

M. kistensis and *M. lamarrei* are on record[3] whose nutritional value is at par with other culturable fish species[4]. Prawn and shrimp farming requires suitable candidates which can withstand captive conditions, has higher genetic diversity and genetic variability to adapt to different environments. In this regard the extent of variability needs to be screened through RAPD and ISSR studies and thus extraction of Genomic DNA is prerequisite for any DNA based investigation[5].

The protocol for DNA extraction must be simple, inexpensive, reliable, quick and safe with minimal risk for the user[6, 7]. For PCR amplification quality of the Genomic DNA is crucial as excess of cell debris and proteins may inhibit the amplification process[8]. That's why efficient DNA isolation methods have been a core element of molecular research. In the current study, three protocols such as organic, inorganic and kit method were evaluated and compared for total DNA isolation from *Macrobrachium sp* of Jammu waters.

MATERIAL AND METHODS

Specimens of prawns were collected from Sehi stream (32º 30' N, 74º 43' E) of Jammu district and Kheri stream of Samba district (32º 37' N, 74º 52' E) by using cast net of mesh size 5mm x 5mm and brought to Animal Cytogenetics lab of Department of Zoology, University of Jammu. The live specimens were immediately used for DNA isolation and dead specimens were preserved in 75 % ethyl alcohol.

50 mg of cephalothoracic tissue and 50 mg of muscle tissue of pleopods from fresh as well as alcohol preserved specimens were used for extraction of total DNA. Before homogenizing the tissue, ethyl alcohol was removed to prevent destabilization of the isolated DNA. Three methods of DNA isolation were used and compared for their yield.

Protocol 1. Total DNA was extracted using Salting out method with slight modifications[9]. 50 mg of tissue from each sample was homogenized using mortar and pestle. It was then transferred to microcentrifuge tubes with cell lysis solution containing 10 mM Tris-HCl (pH 8.0), 100 mM EDTA (pH 8.0), 2% SDS (Sodium dodecyl sulphate) with pH 8.0, 0.5 M NaCl. Then 5 µl of proteinase K (20 mg/ml) was added in each tube. The samples were incubated at 60 ºC for 10-12 hours (with periodic mixing). After that 6M NaCl (saturated NaCl) was added to each tube and subjected to centrifugation at 8000 rpm for 10 minutes. The supernatant was collected and transferred to other microcentrifuge tube. DNA was then precipitated using absolute alcohol. The DNA pellet was dissolved in 200 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA).

Protocol 2. Genomic DNA was isolated using standard Phenol-Chloroform method with minor changes[10]. After homogenization of 50 mg of tissue, each sample was exposed to the treatment of lysis buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 100 mM NaCl, 1 % SDS (pH 8.0), 5M NaCl. Samples were then incubated with proteinase K (20 mg/ml) at 55 ºC overnight and the lysate was centrifuged at 10,000 rpm for 10 minutes and washed with phenol: chloroform: isoamyl alcohol (25:24:1). In the supernatant the DNA was precipitated with chilled isopropanol and mixed by inversion. DNA pellet was then washed with 70 % ethanol and air dried. After that the extracted DNA was dissolved in 200 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA).

Protocol 3. The DNA was extracted using kit method (DNeasy Blood & Tissue Kit, Qiagen, Germany) following the Kit- manufacturer's instructions with modifications. To about 25-50 mg of tissue from each sample in a 1.5 ml microcentrifuge tube, Buffer ATL (Lysis buffer) was added along with 20 µl of Proteinase K and incubated at 56 ºC for 10-15 minutes until completely lysed. Then after few treatments with other buffer solutions, the DNA was finally eluted on a spin column membrane.

Evaluation of DNA purity, Quality and Quantity

Agarose gel electrophoresis is a standard method to determine the quality of Genomic DNA as it separates and recognizes DNA fragments according to their molecular weights. 1 % Agarose gel was prepared to check the total DNA of prawns. Clear and sharp bands near the wells indicated high molecular weight DNA. The agarose gel was then photographed with high resolution camera. The quantity of Genomic DNAs isolated by three protocols were determined by taking the absorbance reading at wavelength of 260 nm on UV spectrophotometer and the purity of DNA

was analysed by calculating the ratio of sample absorbance at 260 and 280 nm (260/280)[11,12,13]. The DNA concentration (C) was determined following the formula: Concentration (C) = $A_{260} \times 50\ \mu g ml^{-1} \times$ dilution factor[12, 14,15]. A 50 $\mu g ml^{-1}$ solution of double stranded DNA gives the optical density reading of 1.0 at 260 nm[11].

Fig. 1a. *Macrobrachium dayanum*

Fig. 1b. *Macrobrachium kistensis*

Amplification by PCR

The Genomic DNA isolated by three protcols were subjected to PCR amplification by RAPD (5'-*CAGGCCCTTC*-3') and ISSR (5'-*CACACACACACACACAAT*-3') primers. The polymerase chain reaction was performed using 2 µl DNA, 2.5 µl reaction buffer, 1.0 µl dNTPs (10 mM), 1.0 µl Taq Polymerase (1U/µl), 2.5 µl $MgCl_2$ (25 mM), 2.0 µl primer and 14 µl PCR water to make up the final rxn volume of 25 µl. The conditions used for the amplification were as follows: an initial denaturation step at 95°C for 5 minutes; followed by 45 cycles of 94°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute; and finally elongation (extension) at 72°C for 10 minutes.

The results were presented as means ±SD and were processed using Statistical Package for Social Sciences (SPSS) software version 20.

RESULTS AND DISCUSSION

Among the three studied species of prawns

Fig. 1c. *Macrobrachium lamarrei*

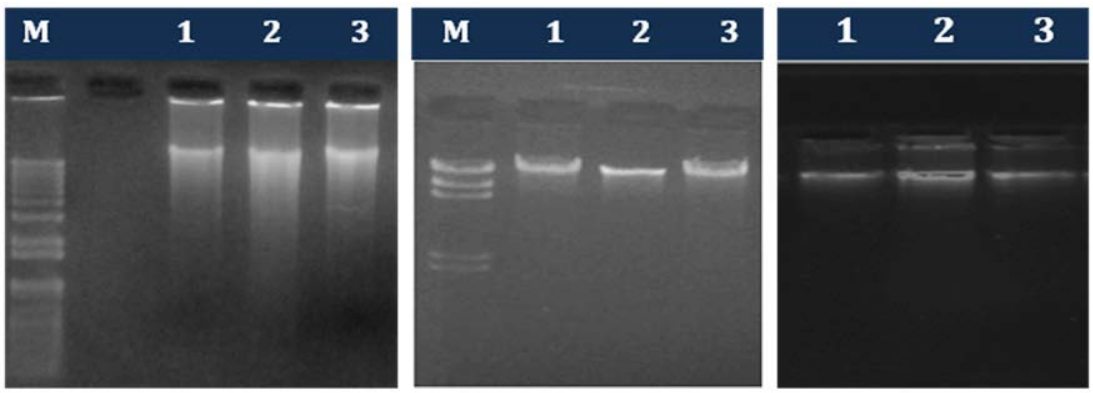


Fig. 2. DNA isolated by three methods; 2a: Salting out, 2b: Phenol-Chloroform method, 2c: Kit method (Lane M represents DNA ladder, Lane 1,2 and 3 depicts DNA of three species of *Macrobrachium*)

(Fig. 1a, 1b, 1c) *Macrobrachium dayanum* yielded high quality DNA in all the extraction protocols. DNA concentration, however, in two species viz., *M. dayanum* and *M. kistensis* was observed to be highest following the Phenol-Chloroform method as compared to salting out and kit method where as in third species *M. lamarrei* high concentration DNA was extracted by kit method as depicted in Table 1. A concentration range between 180 to 315 µg/ml, 67.5 to 752.5 µg/ml and 42.5 to 365 µg/ml was found using salting out, phenol-chloroform and kit methods respectively, thereby revealing phenol-chloroform method to be the best for DNA extraction of fresh water prawns. The purity of

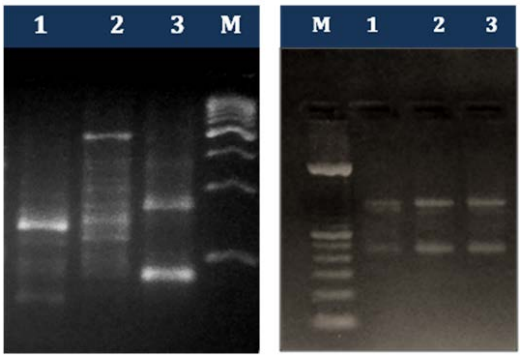


Fig. 3. PCR amplification, 3a: By RAPD primer, 3b: By ISSR primer

Genomic DNA isolated by three protocols was determined by ratio of optical density readings at 260 nm and 280 nm on UV spectrophotometer as shown in Table 2.

The high quality DNA isolated from above methods was subjected to PCR amplification by RAPD and ISSR markers. The electrophoretic pattern of genomic DNA showed single sharp and distinct band on 1 % agarose gel for each sample and the electrophoretic images of PCR products showed many sharp and distinct bands on 1.8 and 2 % agarose gel for prawn populations as depicted in figure 2 and figure 3.

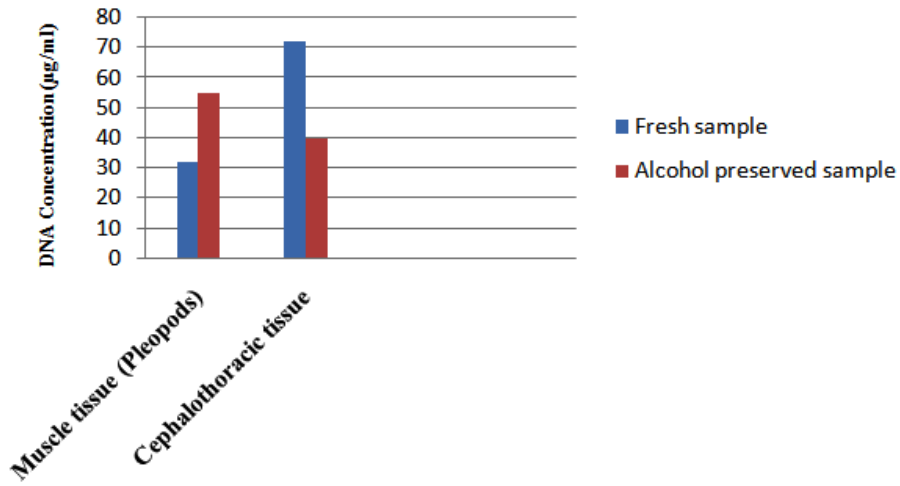
The two different tissues *viz.*, cephaothoracic tissue after removal of carapace and muscle tissue of pleopods were used for isolation of DNA. The quantity of DNA in cephalothoracic tissue was higher compared to muscle tissue in the freshly collected prawn samples whereas the DNA concentration was higher in muscle tissue than the other tissue as shown in the graph 1. The reason for this can be attributed to the fact that the hepatopancreas in the cephaothoracic region is light orange in colour. It is known to possess numerous chromatins which are supposed to contribute in the absorbance spectrum thereby increasing yield[5]. Regarding the purity and quality of DNA the value of $OD_{260/280nm}$ around 1.8 is considered best[16, 17, 18]. The value of $OD_{260/280nm}$ less than 1.8

Table 1. Mean values of DNA concentration by three protocols

Prawn species	DNA Concentration (µg/ml)		
	Salting out method	Phenol-Chloroform method	Kit method
Macrobrachium dayanum	315±0.77	752.5±0.68	42.5±0.8
Macrobrachium lamarrei	180±1.2	67.5±0.90	335±1.0
Macrobrachium kistensis	215±0.78	540±1.1	365±0.88

Table 2. Mean values of optical density for estimation of purity of DNA

Prawn specimens	DNA extracted by Salting out		DNA extracted by Phenol-Chloroform		DNA extracted by Qiagen kit	
	OD_{260}	$OD_{260/280}$	OD_{260}	$OD_{260/280}$	OD_{260}	$OD_{260/280}$
Macrobrachium dayanum	0.126±0.0005	1.82±0.0008	0.301±0.0006	1.79±0.0005	0.017±0.0009	1.88±0.0005
Macrobrachium lamarrei	0.072±0.0007	1.74±0.0009	0.027±0.0008	1.86±0.0009	0.134±0.0005	1.83±0.0002
Macrobrachium kistensis	0.860±0.0005	1.83±0.0009	0.216±0.0005	1.89±0.0008	0.146±0.0002	1.71±0.0005



Graph 1. Showing the comparative yield of DNA in fresh and preserved samples

specify protein or phenol contamination and value higher than 1.8 indicate RNA contamination[5, 15]. The phenol-chloroform method has also been found high yielding for DNA isolation in shrimps as compared to other two methods [5, 19]. Also, this method is used commonly for extraction of gDNA in studies pertaining to taxonomy and detection of white spot syndrome viruses in several species of prawns and shrimps as well as other crustaceans[20,21,22].

CONCLUSION

The present study depicts that Phenol-Chloroform method is best to isolate DNA from fresh water prawns. The genomic DNA obtained by it provided better PCR results for RAPD and ISSR analysis of genetic diversity of prawns.

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