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Genomic DNA Extraction from Various Tissues of *Prawn Penaeus semisulcatus*: A Modified Procedure

عزل الحمض النووي من أنسجة عينات روبيان أم نعيرة
Prawn Penaeu semisulcatuss

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Abstract: The objective of this study was to adapt a procedure to isolate genomic DNA from the prawn *Penaeus semisulactus* collected from the Arabian Gulf region. Different tissues and organs of the prawn were used for the isolation of biologically active, intact gDNA. Several attempts to isolate DNA from the prawn *Penaeus semisulcatus* using previously reported procedures proved of limited success. Subsequently, a modified protocol for the extraction of total cellular DNA from various tissues of the prawn was developed and optimized. Here, it is demonstrated that the modified DNA isolation procedure is suitable for *Penaeus semisulcatus* species, and yields intact, pure gDNA that is suitable for molecular studies.

Keywords: *Penaeus semisulactus*, Tissues and organs, molecular, genomic, DNA, Arabian Gulf, Kuwait.

المستخلص: تهدف هذه الدراسة لإيجاد أفضل الطرق لعزل الحمض النووي من روبيان أم نعيرة *Penaeus semisulactus* والذي تم تجميع عينات منه من مناطق مختلفة من الخليج. استخدمت عدة أنواع من أنسجة الروبيان لاستخلاص حمض نووي متكامل وباستخدام عدة طرق للعزل، وقد تم تطوير طريقة تمثل الأفضل لهذا النوع من الروبيان، وهذه الطريقة تعتمد على توفير حماية أفضل للحمض النووي من التحلل الذي كان ينتج عند استخدام الطرق الأخرى.

كلمات مدخلية: روبيان، الخليج العربي، أنسجة، حمض نووي، حماية، تحلل

Introduction

Penaeus semisulactus, is the most important prawn species in Kuwait and the Arabian Gulf countries. It has immense economic value. The species comprises over 90% of the industrial catch, and 37% of the artisanal catch in Kuwait (Mohammed, *et al.* 1979); (Al Attar and Ikenone, 1979); (Enomoro, 1971). Kuwaiti Bay appears to contain important spawning grounds for several penaied species; however, it remains unclear as to whether the spawning stocks are part of, or isolated from, the Gulf prawn stock in general (Al Attar, 1981). Fluctuations in prawn landing have been reported to vary dramatically, which poses the question of population origin.

Whether or not Kuwait's near record landings in 1983/84, and record landings since, have been supplemented with prawns of Saudi origin has been a subject of speculation, and has cast doubt on the accuracy of stock-recruitment relationships. Siddeek and Abdul-Ghafar, (1989), attempted to determine the stock-recruitment relationship, but, in doing so, had to assume that all Kuwaiti prawns had originated wholly from Kuwaiti nursery grounds. Non-random immigration of adult, or sub-adult prawns, would invalidate that assumption.

Based on both the geographical status and the shrimp's life cycle, it is almost certain that *Penaeus semisulactus* populations in the Southern Kuwaiti and Northern Saudi Arabian waters belong to the same stock. However, evidence for such an assumption is lacking. To investigate the mixed stock, it was suggested that efforts be made to

determine stock interaction by tagging through genetic means.

Although tagged and released prawns would provide incontrovertible evidence of stock mixing, political considerations and logistics have precluded such experiments. In addition, the capture, tag-release, and recapture procedures are time and labor consuming. Other techniques, such as protein analysis, are commonly used to determine the authenticity of an individual organism. However, they suffer from the lack of a high degree of individual specificity. On the other hand, comparative examination of morphological characters is well documented as being highly sensitive to environ-mental variation.

The assessment of parameters, such as the amount of genetic diversity within a population, and the amount and kind of genetic differentiation among populations throughout the species range, is a basic requirement of any genetic approach to commercial fishing and aquaculture. Information concerning stock heterogeneity is vital to the design and implementation of adequate management, and for conservation programs. On the theoretical side, knowledge of the nature of genetic differentiation in aquatic species is useful for understanding the patterns of evolutionary divergence.

Fast, accurate authentication of different but similar species is becoming increasingly important for epidemiological and ecological studies. Therefore, new DNA-based differentiation techniques have been introduced, such as finger-printing or genotyping. To establish such molecular techniques, it is vital to have biologically active, pure DNA of good quality for use for restriction digests, legations and PCR reactions. Consequently, the laboratory has developed a simple and efficient procedure for the extraction of high-quality DNA from prawn tissues.

Materials and Methods

(1) Sample source:

Samples of the prawn *Penaeus semisulactus* were obtained from different waters of Kuwait, and Gulf Cooperation Council (GCC) countries, namely Bahrain, Qatar, Saudi Arabia and the United Arab Emirates. The fresh samples were collected from each station, in Kuwait, (Fintas, Umm Al Maradem and Kuwait Bay), and immediately immersed in 100% ethanol, or dry ice, for preservation. Prawns or samples collected from other GCC countries were received within 3-5 days of catching in dry ice and were maintained at temp. (-80°C).

II. Extraction of Genomic DNA gDNA.

Several previously reported procedures were followed to recover a maximum yield of high molecular weight DNA, devoid of proteins, polysaccharides, polyphenalic compounds, and other restriction-enzyme inhibitors, (Tassankajon, *et al.* 1997); (Plumbi and Benzie, 1991); (Garcia and Benzie, 1995); (Al Janabi and Martinez, 1994); (Sambrook, *et al.* 1989); (Bouchon, *et al.* 1994). These techniques were used to extract the gDNA from different tissues of the specimens, such as ovaries, muscles, the hepatopancreas and pleopods.

The tissue samples were either homogenized electrically with a polytron tissue homogenize *(Brinkmann Instrument)* for 10-15 seconds, with different intervals, containing homogenization buffer specified by the procedures, or manual ground using a prechilled mortar and pestle.

Due to gross contamination with orange and the lack of pigmentation experienced in the gDNA, extracted from hepatohancreatic tissues of prawns from the Unted Arab Emirates and Kuwait, respectively, the previously mentioned procedures, the protocol by Garcia and Benzie (1995), was modified to include 3%-5% Polyvinylpyrolidone (PVP) (Loomis, 1974) either alone, or in combination with 100mM DTT.

The pigmentation of the gDNA samples was reduced drastically with the use of 3%-5%, PVP in the reaction buffer, which is reflected in the improvement of the Optical Density (OD) 260/280 readings. A relatively pure gDNA was obtained with an OD260 (280=1.79) (Table 1). However, it was partially degraded and cannot be cut with restriction enzymes. The degradation of gDNA was continually experienced with all the procedures of extractions.

Table (1): Photometric OD Reading for gDNA Reflecting the Quality of the (DNA). Extracted from Penaeus semisulcatus.

Tissue	Location	Treatment	A260	A280	A260/ A280
Hepatopancreas	UAE	-	0.47	0.28	1.66
	UAE	3% PVP	0.48	0.27	1.79
	UAE	5% PVP	0.48	0.27	1.79
	Kuwait Bay	5% PVP	0.017	0.012	1.4
Muscles	Fintas	-	0.24	0.21	1.1
	Umm Almaradem	-	0.014	0.016	0.88
	Kuwait Bay	-	0.011	0.15	0.74
	Fintas	-	0.99	0.88	1.0
	Saudi Arabia	-	0.09	0.08	1.0
Ovaries	Fintas	-	1.5	0.77	1.9
	Kuwait Bay	-	0.6	0.3	1.9
	Kuwait Bay	-	1.5	0.9	1.7

III. The modified procedure

Pleopod tissue was used in this protocol and shown to be the best source of intact gDNA. Homogenization buffer 100 mM Tris- HCL, pH 9.0, 100 mM EDTA, pH 8.0. 1% SDS, and 100 mM NaCL, was added to a tissue sample, 1ml of buffer for 50-70mg of tirrue. The homogenized sample was incubated for 60min at 70°C water bath, after which 250ml of 5M KOC (potassium acetate) was added to give a final concentration of 1M, and the sample was incubated in ice for 30min. DNA was precipitated from the supernatant by 0.8vol isoporonal, and rinsed briefly in 70% ethanol. The DNA was resuspended in TE, pH 7.5.

Pure and intact genomic DNA was obtained when the homogenization buffer was modified to contain 100mM tris-HCL, pH 9.0 and 100mM EDTA, pH 8.0, whereas in the previous other procedures, the EDTA concentration varied in the range 10-50mM. Genomic DNA from fish liver, prawn muscle and pleopod tissues had an OD260/280 of > 1.8 (Table 2) and the DNA was biologically active and pure enough to be used for polymerase chain reactions (PCR).

Table (2): OD Reading of DNA upon increasing the concentration of Tris and EDTA from 10 mM Tris, (pH 7.5 to 100 mM, EDTA to 100 mM Tris, pH 9.0 and 100 mM, EDTA.

Sample	A260	A280	A260/A230
fish liver	0.35	0.185	1.88
Shrimp muscle	0.083	0.030	2.7
Shrimp pleopod	0.066	0.029	2.2

Results and Dissection

In this study, several modifications of the widely used procedures were needed to isolate biologically active, pure, high molecular weight DNA. Hepatopanceratic tissues from *Penaeus semisulactus* samples from Kuwait and United Arab Emirates yielded pigmented gDNA extracts that were black and orange in color respectively, following preparation using the methods of Tassankanan, et at. (1997) and Plumbi and Benzie (1991). The pigmentation is thought to be due to the different diets of prawns, which could include polysaccharides and polyphenolic compounds in the samples.

The pigmentation was decreased drastically with the use of 3-5% PVP, in the procedure of Garcia and Benzie (1995). The gDNA obtained following the protocol of the Commercial Wizard kit (Promega), was partially intact with a smeary tail. When the genomich DNA was checked after a few days of storage at 4°C, it was fully degraded. In the same experiment, fish liver yielded a more pure, intact gDNA under the same storage conditions (Fig. 1). The poor quality of the genomic DNA of prawns remained the same even after CsCl gradient purification (Sambrook, *et al.* 1989).

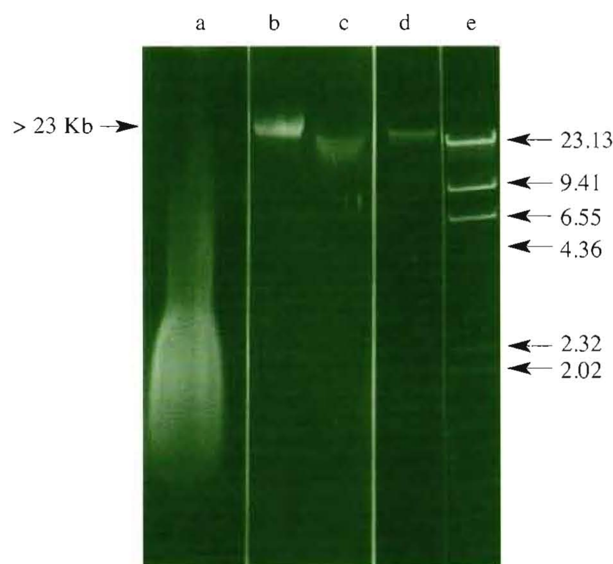

Fig.(1): Genomic GNA extraction using the wizard kit versus the new protocol.

All gDNA are in excess of 23 Kb in size.
- ***(a)*** *(DNA from shrimp muscle tissue showing degradation.*
- ***(b)*** *DNA from fish liver tissue.*
- ***(c)*** *DNA from shrimp muscle tissue.*
- ***(d)*** *Intact DNA from shrimp pleopods extracted using the modified procedure.*
- ***(e)*** *Hind III λ marker.*

Degradation of genomic DNA was also experienced by Benzie (1998) personal communication, and it is thought that the DNA degradation might have happened in the tissues before the DNA was isolated, or after long storage of DNA in TE, even at – 20°C. For such samples, new fresh extracts were prepared. Fresh extracts of mature ovary tissue were capable of degrading Lambda DNA, an observation by Benzie, (1998) which is in agreement with our findings.

However, it appears that storage is not a factor in DNA degradation, at least in samples obtained from Kuwait and Qatar, which were used within one month of storage. This observation, specially in samples obtained from the other countries, might lead to consider that the degradation factor may involve the time lapse between catching and

analysis, as well as handling of the sample, as the samples were stored for periods longer than a month.

Using pleopod tissues, which were homogenized in a buffer contained in 100mM tris HCL, pH 9.0 and 100mM EDTA, pH 8.0 (See, Fig 1, lane d) the genomic DNA extracted was of a good quality and quantity. High concentration of tris and EDTA may have provided better protection for the gDNA against degradation.

Conclusion

Genomic DNA from *Penaeus semisulactus* is highly unstable and degrades easily. This could be due to certain proteins or enzymes in the tissues that affect its stability during the homogenization and lyses of the cells. Isolation of biologically active pure gDNA is the most important step for further molecular studies. Extraction of gDNA from *Penaeus semisulactus* samples obtained from different waters of Kuwait and the GCC countries requires several modifications to the commonly accepted procedures to overcome the gDNA degradation problem.

In this study, pigmentation was experienced in gDNA from hepatopacreatic tissues, which was reduced by the introduction of PVP. Intact, biologically active, pure gDNA was isolated using high concentrations of tris and EDTA in the homogenization buffer. The resulting DNA appeared intact and free of contaminates, and is suitable for molecular studies.

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