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Research Article

RAPD ANALYSIS FOR GENETIC DIVERSITY OF GREEN MUSSEL (*PERNA VIRIDIS*) IN SOUTH INDIAN COASTAL REGION

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD) is used to determine genetic diversity of the six populations of green mussel (*Perna viridis*) collected from contaminated and uncontaminated coastal environments of South India. The analysis of genetic distance (Dn) among the six populations showed high genetic similarity with a range of Dn values from 0.0355-0.3596. At the species level, the percentage of polymorphism (89.11), Nei's genetic diversity (0.418) and Shannon diversity index (0.273) was observed. The total genetic diversity between populations ranged from (Ht) 0.3378 to 0.4800, the subpopulation genetic diversity (Hs) ranged from 0.3087 to 0.4500. Coefficient of overall differentiation (GST) between populations ranged from 0.0358 to 0.2404. Indirect estimate of the level of gene flow between populations ranged from 1.5798 to 4.5827. These prominent results might explain genetic diversity observed within each population and the moderate genetic differentiation among populations. Thus this study suggests that RAPD marker could be helpful in providing information about genetic diversity of the population.

Key words: Genetic diversity; RAPD-PCR; *Perna viridis;* Mussel;Biomonitoring; Markers.

INTRODUCTION

Genetic diversity and population distinctiveness is a major goal in the conservation management plans of overexploited species. Genetic data and phylogeographic structure throughout the species' range constitute the basic information required to understand their evolution and biogeographic history (Meffe, 1994). A widespread decline in the mussel population in Indian coastal waters has led to a persistent need to assess genetic variability in the wild population for proper conservation. Marine pollution is caused by the deleterious effect as a result of increased anthropogenic activity, indiscriminate discharge of effluent from industrial, agriculture and domestic sources disposed into coastal environment. Toxic pollutants affect marine biota which circulates into the food chain and might end up as sea food products causing various problems (Reish *et al*., 1993). Naturally living organism can accumulate essential and nonessential heavy metals in their tissue, which can cause deleterious effects by interfering their metabolic activities (Taseli 2009., Mahmoudi *et al*., 2010., Nakane and Haidary, 2010).

Toxic pollutants such as heavy metal causes gamete loss due to the cell death, embryo mortality (lethal mutation), abnormal development, neoplasia, heritable mutation, and reduced reproductive capacity which may be cause for low genetic diversity in the marine organisms (Tice, 1990., Kurelec,1993., Savva,1998). Hence the mussels are considered as a good bioindicator for pollution assessment including survival, behavioural changes, reproductive changes, transplant experiments and genotoxicity (Fowler and Oregioni 1976., Yap *et al*., 2003). Recently, the development of molecular marker technology has provided new tools for detection of genetic alteration in response to heavy metal tolerance by looking directly at the level of DNA sequence and structure (Noel and Rath, 2006., Atienzar and Jha, 2006., Atienzar, 2000., Bensch and Kesson, 2005., De Wolf, 2004). Random amplified polymorphic DNA is a PCR-based technique and extremely efficient for DNA analysis in complex genomes as it is relatively inexpensive and yields information on a large number of loci without having to obtain sequence data for primer design (Bussell,1999., Ouborg *et al* 1999., Chen *et al* 2005., Williams *et al*., 1990). RAPD profiles were achieved by PCR with single short primers of arbitrary nucleotide sequence under low annealing conditions and it is used in surveying genomic DNA to detect various types of DNA damage and point mutations rearrangements, small insert or deletions of DNA and ploidy changes (Nadig *et al*., 1998; Krane *et al*., 1999; Meetu Gupta and Neera Bhalla Sarin, 2009). The present study examines the genetic structuring of a subset of six green mussel population using RAPD assay.

Indian subcontinent has a vast coastline of about 6100 km with Arabian Sea on the west, Bay of Bengal on the east and Indian Ocean towards the south. Most of the industries situated along the coastal region discharge there effluents in to the ocean. This leads to drastic effects over the marine organisms by the process of

biomagnification, bioaccumulation and affect genetic diversity. The present study is carried out along the coastal regions of Tamil Nadu (Cuddalore, Pachayankupam, Chithiraipettai, Mudasalodai, and Rameshwaram) and Kerala (Calicut). The study area map and sampling locations are represented in the (figure 1).

MATERIALS AND METHODS

Marine mussel (*Perna viridis*) samples were collected from different locations along the east and west coast of India. The sample collection sites include SIPCOT (S1), Pachayankuppam (S2), Chithiraipettai (S3), Mudasalodai (S4), Rameshwaram (S5), and Calicut (S6). The collected samples were labelled and kept in ice and then transported to the laboratory and stored at - 80º C until further analysis. At each sampling locations latitude and longitude were determined by Global Positioning System (GPS - Leica GS20).

Genomic DNAs were prepared from dissected adductor tissues, DNA isolation was carried out using a method followed by (Winnepennincks, 1993). Determination of concentration and purity of DNA was obtained with ratio of OD 260 to OD 280 measured with a spectrophotometer. Pure DNA sample were diluted to the final volume of 30 µl with sterile Millipore water. Total genomic DNA samples were analyzed by 2 % agarose gel electrophoresis. DNAs were diluted to appropriate concentration for PCR amplification. About eight primers were arbitrarily tested on approximately twenty individual samples to determine which primers can generate clear bands. Each amplification reaction was performed using a single primer and repeated at least once to verify band auto similarity and repeatability (Perez, 1998). RAPD primers (10 nucleotide length) were used to amplify the random segments of genomic DNA and it is able to differentiate between genetically distinct individuals, although not necessarily in a reproducible way. Three RAPD primers used in the study were (OPB07, OPB10 and OPC09) and the primer details are represented in Table 1. This was used as a marker to analyze the genetic diversity of an individual from the population (*Perna viridis*).

A polymerase chain reaction (PCR) was carried out in total volume of 12.5 µl containing 1 µl of DNA, 1.25 µl of Reaction buffer, 0.4 µl of dNTPS, primers (forward and reverse)1 µl each, 0.25 µl of Taq DNA polymerase and 9.0 µl of Millipore water. PCR amplification were performed with an initial- pre-denaturation for 3 minutes at 94º C followed by 37 cycles of denaturation at 94º C for 1 minute and optimum annealing temperature of 49.5º C for a second and extension at 72º C for 1 minute. The amplification was concluded with a 15 minute final extension at 72º C. The amplified PCR products were separated by electrophoreses in 6% denaturing polyacrylamide gel 40 V for four to five hour in 1x TBE (Tris- Boric acid –EDTA) buffer, and stained with silver nitrate. The primer which separated into clear and reproducible bands was used for further experiments. Amplifications were performed at least twice in order to confirm the results obtained. The amplifications result were analyzed using the image analyser, quantity software (Biorad), 1kb DNA ladder was used.

Bands of equal size were interpreted as homologous and staining intensity of bands were not taken into account. Each band in the RAPD profiles was treated as an independent locus with two alleles, based on the presence or absence of a band. Amplification products in the selected population were scored manually, 1for presence and 0 for absence of homologous bands in all other populations. Relationships among RAPD phenotypes were studied following the recommendations of previous study (Lynch and Milligan, 1994). Population genetic parameters such as expected heterozygosities (gene diversity), genetic differentiation, and genetic distance were calculated using corrections for bias in estimating the allele frequencies. Nei's genetic diversity was also measured as the percentage of polymorphic bands (PPB), and Shannon diversity index was calculated for each individual, each population, as well as the mean and overall value for all populations and each primer (Nei,1973., Yeh, 2000). RAPD marker frequencies were used to calculate the within population gene diversities (Hs), the total gene diversity (Ht) and the coefficient of genetic differentiation among populations (Gst) according to the formula by a previous study (Nei,1978) and unbiased genetic identity and the genetic distance were estimated. All the above analyses were performed using POP GENE software (Vers.1.32).

RESULTS

Among the ten investigated primers, three primers were found to be amplified successfully with reproducible results and were selected for the analysis of genetic diversity and identification of molecular markers of mussel (*Perna viridis*). Screening of selected primers showed polymorphisms within the six populations, Primers OPB-7, OPB-9, and OPB-10 produced 6, 11 and 8 bands respectively ranging from 200 to 2000 bp. Thus in total, 21 bands were scored for presence (1) and absence (0). The analysis of Nei (1978) unbiased genetic distance (Dn) among the six populations of *Perna viridis* showed high genetic similarity with a range of Dn values from 0.0355-0.3596 and the data were presented in Table 2. At the species level, the percentage of polymorphim (PPB), Nei's genetic diversity and Shannon diversity index were 89.11%, 0.418 and 0.273 respectively. Within the populations, the PPB ranged from 60.25 to 87.12%; Nei's genetic diversity between the populations ranged from 0.1764 to 0.2630, and the Shannon diversity index between the populations ranged from 0.2671 to 0.3841. The total genetic diversity between populations (Ht) ranged from 0.3378 to 0.4800. The subpopulation genetic diversity (Hs) ranged from 0.3087 to 0.4500. Coefficient of overall differentiation (GST) between populations ranged from 0.0358 to 0.2404. Indirect estimate of the level of gene flow between populations was calculated, and the gene flow (Nm) value ranged from 1.5798 to 4.5827 individuals per generation as shown in Table 3.

DISCUSSION

Genetic variation in different populations of a biological species is an important strategy towards the assessment of pollution and its impact on the species diversity over time. According to previous

report (Ward, 2006), the variations in genetic level in invertebrates strongly depend on environmental stress, geographic range and larval dispersal mechanism. RAPD profiles detect alterations in genomic DNA with the use of arbitrarily primer PCR reactions and clearly show the detection of pollutant-induced DNA damage (Enan, 2006., Li Ma, 2000). Hence, the PPB, Nei's genetic diversity and the Shannon diversity index were all important parameters to assess the variation within population. PPB is calculated for the proportion of polymorphic bands, and only genetic polymorphism is considered; whereas in Nei's genetic diversity, both polymorphism and gene heterozygosity were considered. Thenceforth, the Shannon diversity index is calculated for each locus and averaged over loci to provide the variation within populations.

In *P. viridis*, PPB, Shannon diversity index and Nei's genetic variations was recorded high in S1 of about 87.12%, 0.3668, and S6 0.2630 respectively at the population level. The similar genetic diversity of prawn population near smelter discharging site was lower than that of the uncontaminated site (Ross and Bidwell, 2003., Qian, 2001). The high genetic variability was found in SIPCOT industrial area due to polluted environmental conditions without affecting the species diversity of the population. Due to long term exposure of various chemicals from industries, survival of bivalves depends on the level of genetic variability within populations (Dawson, 1995).

Normally Shannon's diversity index is well suited for RAPD analysis but relatively becomes insensitive when heterozygous individuals are measured (Slatkin, 1987). The levels of Nm>1 are sufficient and are thought to prevent genetic difference among the population (Pereira, 2008). Hence Nm = 1.8854 > 1.5798 in the region of SIPCOT and higher than Calicut were due to the larval exchange and their genetic similarities with reference to the population prevailing in that area (Fernandez-Tajes, 2007). The previously obtained Nm, 2.9 between two populations (*R. Decussates*, also *E. Siliqua)* have reported the same gene flow from the same magnitude among Galcian populations of their study (Apte, 2001). The RAPD results reveal that the difference between gene flow from SIPCOT industry is low, where as the gene flow of other are higher. Although there is less geographic distance between the samplings sites of SIPCOT, Pachayankupam, Chitraipettai, Mudasalodai and Rameshwaram, the Gst values are around 0.2404, 0.0358, 0.0984, 0.1171 and 0.1016 respectively. Differentiation was found in the populations between the Calicut (0.2096) due to varying geographic distances of about 850 km approximately along the coastal region of South India. The genetic differentiation obtained in the study was in contrast to previous allozyme study which also failed to exploit the genetic heterogeneity among the same population (Savva, 1994). The genetic distance between the six populations of *Perna viridis* have shown a wide variation (0.24233 – 0.3033). Based on the geographic location, SIPCOT lies in the South-east coast of India, while the Calicut region lies in the South-west coast. Increased industrial, anthropogenic and other source of pollution could interfere

in the genetic variation in this region. Based on the results, RAPD markers can determine the alteration in the genomics due to negative induction of marine pollutants (Liu, 2004). Genetic diversity evolutionarily helps to adapt to environmental changes and stressors within a population.

CONCLUSION

Genetic diversity data for the selected populations can be effectively used to identify the individual's possessing high differentiation for future perspectives. The results of this study show that the DNA polymorphisms detected by RAPD analysis can be applied as a suitable biomarker assay for the detection of genotoxic effects on mussel *(Perna viridis),*and this can be used as a biomonitoring tool in toxic marine environment. Further research is required to better understand the potential of the RAPD assay for the detection of DNA damage and mutations.

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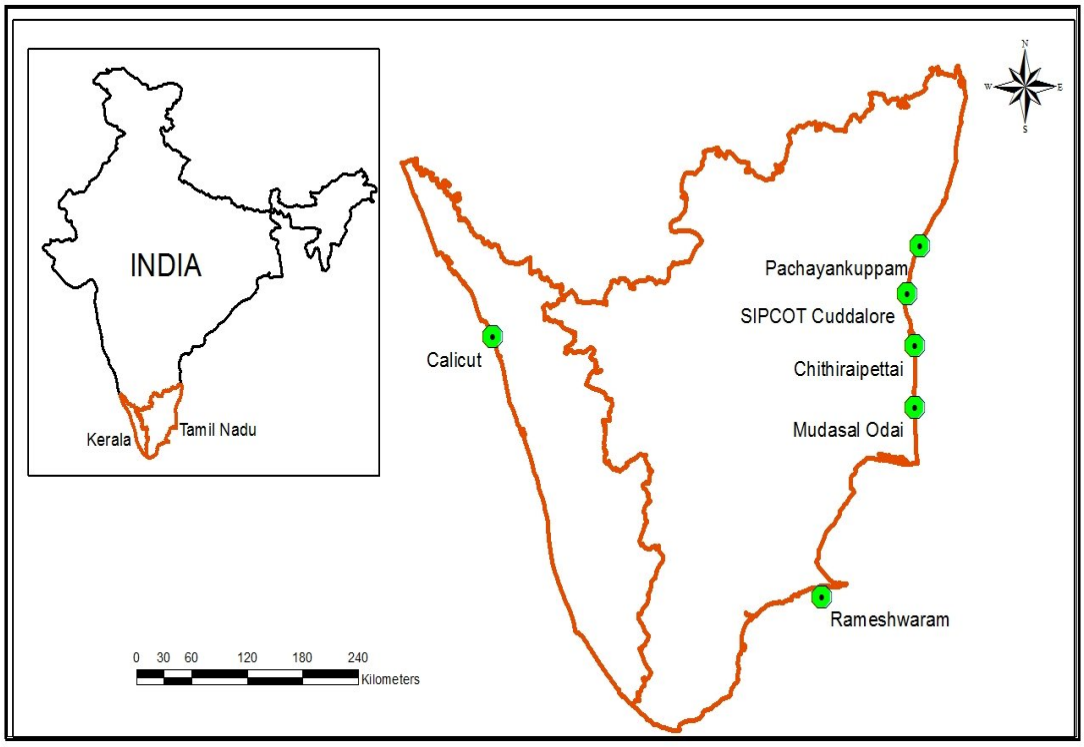

FIGURE 1. STUDY AREA MAP

TABLE 1. RAPD PRIMERS USED

S.No	Primer code	Primer Sequence
1.	OPB-07	GGT GAC GCA G
2.	OPB-10	CTG CTG GGA C
3.	OPC-09	CTC ACC GTC C

TABLE 2.GENETIC SIMILARITY INDICES AND GENETIC DISTANCES AMONG SIX POPULATIONS OF MUSSEL *(PERNA VIRIDIS)*

Population	**S1**	S2	S3	S4	S5	S6
S1	*****					
S2	0.1814	*****				
S3	0.1533	0.3596	******			
S4	0.1328	0.1227	0.1523	******		
S5	0.3033	0.1554	0.2552	0.1127	******	
S6	0.0355	0.2016	0.2557	0.3455	0.2413	******

TABLE 3. GENETIC VARIABILITY OF SIX MUSSEL (*PERNA VIRIDIS*) POPULATION

Population	**H**	**I**	**Ht**	**Hs**	**Gst**	**Nm**	**PPB**
S1	0.2516±0.18	0.3841±0.22	0.4800	0.4012	0.2404	1.8854	87.12
S2	0.2329±0.18	0.3362±0.27	0.4439	0.3624	0.1171	3.7681	71.21
S3	0.2170±0.20	0.3515±0.28	0.4487	0.4215	0.1016	4.4233	70.10
S4	0.2149±0.21	0.3241±0.29	0.3775	0.3624	0.0984	3.482	64.35
S5	0.1764±0.18	0.2671±0.27	0.3378	0.3087	0.0358	4.5827	60.25
S6	0.2630±0.16	0.3668±0.25	0.4666	0.4500	0.2096	1.5798	79.26
Mean	0.2259	0.3381	0.4259	0.3843	0.1338	3.2866	72.02

Source: Compiled by Author

Note: N - Sample size**H -** Nei genetic diversity, **I -** Shannon diversityindex , **Ht -**Total genetic diversity, **Hs -** Genetic diversity within population, **Gst -** Genetic diversity among populaton, **Nm -** Gene flow, **PPB -** percentage of polymorphic band.