Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Research Paper

Preliminary Investigation of Genetic Heterogeneity of Some Indian Freshwater Mussels Using Rapd Markers

Zoology

KEYWORDS : RAPD, Polymerase Chain Reaction, Electrophoresis, Genetic diversity, Molecular Markers

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ABSTRACT

India has a widespread distribution of freshwater bivalve mussels which have ecological and economical importance. Use of shell pattern and morphological characteristics for identification of these mussels could lead to error. The present study reports a preliminary investigation of genetic diversity evaluation in these bivalves collected from the State of Maharashtra using RAPD makers. Genomic DNA was isolated and amplified with twelve different RAPD primers which generated amplification fingerprint specific for each species under the study. Different amplification patterns by a single marker in different samples indicated the polymorphism.

INTRODUCTION

India is blessed with a rich molluscan diversity and is reported to have 52 species of freshwater bivalve mussels (Subba Rao, 1989). These mussels have commercial applications as some of these are used in pearl culture operations, ornament, button making and cosmetic industries. Some are also known to have medicinal properties whereas some are used as food and feed (Subba Rao, 1993; Prabhakar and Roy, 2009). These bivalves also serve as indicators of ecological health. A basic problem with these freshwater mussels is that many a times, their identification is very confusing. Identification is normally based on morphological characteristics which tend to overlap and can create perplexity. So, the use of molecular markers is advocated for reliable species identification and differentiation.

Among the various DNA based markers, Random amplified polymorphic DNA (RAPD) markers have gained considerable attention and popularity in recent years because of their simplicity, speed and cost effectiveness. RAPD analysis involves the PCR amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence. RAPD markers allow creation of genomic markers from species with no prior knowledge about target sequences to be amplified (Hadrys et al., 1992).

RAPD analysis has been employed to evaluate genetic diversity for species, subspecies and population/stock identification in a number of aquatic organisms such as fish, mussels and crustaceans (Barman et al., 2002; Zarattini et al., 2002; Yap et al., 2007; Sleem and Ali ,2008; Rozhkovan et al., 2008; Visvanathan et al., 2014). In India, the use of RAPD markers for genetic diversity studies of bivalve mussels is still in the preliminary stages with only a couple of reports available (Jadhav and Jamkhedkar, 2009; Upadhye et al., 2011). Therefore, an attempt has been made to evaluate the use of RAPD markers to analyze and reveal comparative genetic diversity of three different freshwater mussels collected from the same river of the state of Maharashtra.

MATERIALS AND METHODS

25 healthy specimens each of freshwater bivalve mussels *P. corrugata*, *L. marginalis* and *C. regularis* were manually collected from the Godavari river from Nanded district of Maharashtra. Collected samples were brought alive in separate plastic containers having holes for aeration to the laboratory of the Department of Zoology, Kirti M. Doongursee College, Mumbai and acclimatized for 48 hr in separate glass aquaria in order to overcome the possible transportation stress. After acclimatization, 10 active mussels were sacrificed and the mantle tissue was carefully dissected and separated. These freshly dissected body parts were subjected to isolation of genomic DNA.

1) Isolation of Genomic DNA

The genomic DNA was isolated from the mantle tissue following the protocol standardized by Upadhye et al., (2011). Agarose gel electrophoresis (0.8% agarose) was carried out to check the presence of DNA. Spectrophotometric analysis of the isolated genomic DNA was also carried out at wavelengths 260 and 280 nm to check for the presence of any possible protein or RNA contaminations.

2) Amplification of Genomic DNA using PCR

The isolated genomic DNA was subjected to polymerase chain reaction (PCR) amplification with twelve random 10-mer primers (originally designed by Operon Technologies, USA and synthesized by MWG Biotech, Bangalore) (Table 1). The genomic DNA was amplified in a 50 µl reaction mixture containing 1µl (100 ng/ µl) genomic DNA as template, 25 µl PCR master mix (Fermentas, USA), 2 µl primer (concentration 10 pM) and 22 µl of nuclease free water (Table 2). DNA thermal cycler of the make Biometra, Germany was employed for DNA amplification. PCR conditions were as follows: - First cycle of 5 min at 94°C for template denaturation, followed by 40 cycles of 1 min at 94°C, 1 min at 34°C, 2 min at 72°C. An additional cycle of 7 min at 72°C was used for final primer extension. Amplified products were analyzed by electrophoresis on 1.4% agarose gel.

3) Agarose gel electrophoresis

For genomic DNA 0.8% agarose gel was used whereas for PCR product 1.4% agarose gel was used. DNA samples or PCR products (10 µl of the sample with 2 µl of the gel loading dye) were added to the sample wells in the agarose gel using a micropipette. DNA ladders of different sizes (5 µl) (SM1143 and SM0311 Fermentas, USA) were loaded in first and the last well. Electrophoresis was carried out at 85mA and 180 volts for approximately 1 hr by observing dye front. The gel was stained in solution containing 5 µl of ethidium bromide in 50 ml of distilled water for 15-20 min and observed under UV transilluminator for DNA bands. Photographs of the gel were taken.

Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Table 1 Details of the twelve RAPD primers used in the study

Name of Primer	Primer sequence
OPA-01	CAGGCCCTTC
OPA-09	GGGTAACGCC
OPA-13	CAGCACCCAC
OPB-01	GTTTCGCTCC
OPB-02	TGATCCCTGG
OPB-08	GTCCACACGG
OPB-16	TTTGCCCGGA
OPD-02	GGACCCAACC
OPD-08	GTGTGCCCCA
OPD-20	ACCCGGTCAC
UBC456	GCGGAGGTCC
UBC457	CGACGCCCTG

Table 2 Composition of the PCR reaction mixture

Component	Volume
PCR Master mix	25 µl
DNA (diluted original DNA to 1:3)	1 µl
RAPD primer	2 µl
Nuclease free water	22 µl
Total volume	50 µl

4) Analysis of RAPD amplification pattern

Photograph of the gel was captured under UV and saved on computer. Using Microsoft power point the report was prepared with the photograph, and the table for primers as well as the individual band size was prepared with reference to the ladder. Using this table, data were analyzed for common bands (similarity) and polymorphic bands (diversity).

FINDINGS

Out of the twelve primers tried for RAPD-PCR, six RAPD primers showed amplification and DNA fingerprint for minimum one sample as shown in the Table 3.

Primer OPA-09 generated a total of 11 amplification bands in which only a single fingerprint at 680 kD was generated for *P. corrugata* whereas *C. regularis* and *L. marginalis* produced 5 amplification fingerprints each. However, these 5 fingerprints were of different molecular weights indicating polymorphism.

Primer OPA-13 also gave rise to a total 11 amplification bands in which 3 bands of different sizes were produced for each *P. corrugata* and *C. regularis* whereas *L. marginalis* gave rise to 5 bands, four out of these 5 were found to be polymorphic. OPA-13 generated a common band of 720 kD in both *L. marginalis* and *C. regularis* indicating some similarity between the two species.

Compared to other primers, Primer OPB-01 produced least number of amplification fingerprints with only a single band of 1700 kD for *P. corrugata* and 2 bands of comparatively lower molecular weights for *C. regularis* and no amplification in case of *L. marginalis*.

Primer OPD-02 produced 4 bands each for *C. regularis* and *L. marginalis*. All these bands were of different weights indicating polymorphism. Primer OPD-02 failed to generate any amplification fingerprint for *P. corrugata*.

Primer OPD-08 generated a total of 9 amplification bands in which 5 fingerprints were generated for *L. marginalis* whereas *C. regularis* and *P. corrugata* produced 2 amplification fingerprints each. Again, except a common band of 520 kD for *P. corrugata* and *C. regularis*, all other fingerprints were of different molecular weights indicating polymorphism.

Primer OPD-20 generated a total of 8 amplification bands in which *C. regularis* and *P. corrugata* produced 3 amplification fingerprints each. Two fingerprints were generated for *L. marginalis*. Even these fingerprints were of different molecular weights indicating polymorphism.

Primers UBC- 456, UBC- 457, OPA-01, OPB-02, OPB-08 and OPB-16 failed to amplify any target DNA of the studied animals.

Table 3 Amplification fingerprint generated in study animals by six RAPD primers

Primer	Parreysia corrugata	Corbicula regularis	Lamellidens marginalis
OPA- 09	680	430, 500, 660, 800, 1150	400, 650, 720, 1000, 1350
OPA-13	540, 630, 760	720, 1200, 1450	720, 850, 1000, 1750, 2500
OPB-01	1700	580, 680	No band
OPD-02	No band	1100, 820, 630, 680	1250, 800, 560, 330
OPD-08	520, 1400	460, 520	480, 580, 650, 860, 1500
OPD-20	460, 730, 1000	450, 1000, 1150	380, 650
	Size of amplification fingerprints (mol. Wt in kD)		

CONCLUSIONS

In spite of being a preliminary investigation, the present study clearly suggests that RAPD markers can be used for species differentiation and for studying genetic diversity. More studies are ongoing with more number of diverse primers to target and explore more genomic DNA and to get more precise results. After generation of large data, it will be analyzed with standard softwares of population genetics to find the exact phylogenetic distances between these species and construct a phylogenetic tree.

ACKNOWLEDGEMENTS

Authors would like to express their sincere thanks to Dr. M. V. Upadhye, Mumbai and Dr. A. N. Thakur, Goa for their valuable help and support during the course of this study.

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