ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (2): 338-342, 2008
ISSN 1682-296X

Genetic Characterization of Wild Stocks of Prawns *M. rosenbergii* using Random Amplified Polymorphic DNA Markers

[1]L.M. See, [2]R. Hassan, [1]S.G. Tan and [2]S. Bhassu
[1]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia
[2]Freshwater Fisheries Research Center, Glami Lemi, 71650 Titi, Jelebu, Malaysia

Abstract: Random amplified polymorphic DNA was used to study the genetic diversity and assay polymorphisms among eleven populations of *Macrobrachium rosenbergii* in Malaysia. The RAPD procedure generated 205 markers in the 11 populations. and a large level of polymorphisms was observed in all populations. The unweighted pair group method with arithmetic averaging (UPGMA) clustering procedure based on the markers identified three major clusters among all populations. The results of the present study showed that RAPD based fingerprinting were a useful tool to assess the genetic variability of freshwater prawn, *M. rosenbergii*. Assessing high genetic variability within and among *M. rosenbergii* is considered important genetic resources as it has direct benefit for conserving wild stocks, greater potential for improvement and serves as invaluable resource for different selection criteria, especially when planning breeding or crossbreeding programs.

Key words: *Macrobrachium rosenbergii*, RAPD, LP-RAPD, RAMs, genetic characterization

INTRODUCTION

Over the past 15 years, *Macrobrachium rosenbergii* has been the subject of intensive study for aquaculture. It is native to the whole of the South and Southeast Asian area, including Malaysia, Thailand, Indonesia, Bangladesh, Philippines, India as well as in northern Oceania and in the western Pacific islands (FAO, 2002). It is the most popular freshwater shrimp for commercial culture and became the preferred shrimp culture species in the 1970's when Dr. Shao-Wen Ling discovered methods to raise the baby prawns to the juvenile stage (Mather and de Bruyn, 2003). In Malaysia and Southeast Asia, *M. rosenbergii* has evolved to survive in the brackish water of estuaries and in rivers. In fact, it grows better in freshwater than when it rarely ventures into water which is more than one half seawater.

Random amplified polymorphic DNA (RAPD) is a multi-locus dominant marker system that serves as a popular alternative because it provides multiple markers without any prior knowledge of the DNA sequences. These oligonucleotides serve as both the forward and reverse primers and usually are able to amplify fragments from 3 to 10 genomic sites simultaneously. The variable lengths of the DNA amplified are inherited as classical Mendelian traits (Williams *et al*., 1990) and thus can be used for genetic analysis (Horn *et al*., 1996). However, this reproducibility of the results requires careful optimizations (Yang and Quiros, 1993).

LP-RAPD is sensitive to genetic variation at both the intraspecific and interspecific levels as the length of the primer is longer and the annealing will be more stable at particular priming sites (Gilling and Holley, 1997). Thus, this technique enables PCR amplification to be more selective and be able to distinguish closely between organisms. However, RAMs may reveal a much higher number of polymorphic fragments per primer due to this technique combines several characteristics of RAPD and microsatellite analysis (Fang and Roose, 1997; Esselman *et al*., 1999). The objectives of this study were to assess the genetic relatedness among 11 populations using RAPD as well as the comparison of the genetic marker with LP-RAPD and RAMs.

MATERIALS AND METHODS

Sample collections and DNA isolation: Prawns, *Macrobrachium rosenbergii* were collected from 11 streams in 9 states in Malaysia in between Jun to Aug., 2006. The sampling sites represents a diverse distribution of its habitats in Malaysia. Genomic DNA was extracted from tissues using DNA extraction kit, QIamp DNA Mini (Qiagen, Germany) following manufacturer protocol. The purity of DNA was estimated by ratio of absorbance

Corresponding Author: Dr. S. Bhassu, Freshwater Fisheries Research Center, 71650 Glami Lemi, Jelebu, Negeri Sembilan, Malaysia Tel: 066133050 Fax: 066138352

Table 1: The sequence of 5 primers using for RAPD analysis

Primer	Nucleotide length	Sequence (5' to 3')	GC content (%)	Molecular weight	Annealing temperature, T_A (°C)
OPA1	10 mer	CAG GCC CTT C	70.0	2955	42
OPA3	10 mer	AGT CAG CCA C	60.0	2988	42
OPA4	10 mer	AAT CGG GCT G	60.0	3059	42
OPA9	10 mer	GGG TAA CGC C	70.0	3044	42
OPA10	10 mer	GTG ATC GCA G	60.0	3059	42

reading between 260 and 280 nm using UV spectrophotometer. The quality of DNA was estimated visually on a 0.8% agarose gel.

RAPD assay: A total of 5 OPA primer with 10-mer were used to amplify genomic DNA. Operon 10-mer kits contained 10 base oligonucleotide primers (Table 1) in population genetics study. Samples were amplified in a total volume of 10 μL containing ~20 ng of genomic DNA, 1X PCR buffer, 0.5 mM each of dATP, dGTP, dCTP and dTTP, 50 ρmol of each primers, 2.5 mM of $MgCl_2$ and 1.5 U of *Taq* polymerase (Promega, USA) and deionised water. Amplification were performed in T3 thermal cycler (Biometra) with an initial 3 min predenaturation at 96°C, followed by 40 cycles of denaturation at 96°C for 10 sec, an appropriate annealing temperature for 10 sec and an extension at 72 ° for 30 sec. Lastly, a final extension step at 72°C was performed for 5 min.

Following amplification, the products were electrophoresed on 2% agarose gel in at 78 V in 1x TBE running buffer. The gels were stained with ethidium bromide before being visualized under ultraviolet light.

Data analysis: DNA banding patterns generated by RAPD were scored 1 for bright band and 0 for their absence of each amplified band and allelic frequencies were based on the presence or absence of the bands at each locus. In this study, similarity coefficient was calculated across all possible pairwise comparisons of individuals both within and among the populations using the method of Lynch (1990) with the formula:

$$SI = 2n_{xy}/(n_x + n_y)$$

Where:

n_{xy} = No. of fragments shared by individuals x and y

n_x and n_y = No. of fragments scored for each individual

Pairwise matrix were generated by using RAPDistance version 1.04 (Armstrong *et al*., 1996) and dendrograms were constructed based on the unweighted pair group method with arithmetic averaging (UPGMA) employing the sequential, agglomerative, hierarchical and nested clustering (SAHN) program from Numerical Taxonomy and Multivariate Analysis System version 1.60 (NTSYS-pc software) (Rohlf, 1993). Polymorphism parameters within populations such as the number of polymorphic loci, percentage of polymorphic loci, number of alleles per locus, effective number of alleles per locus and gene diversity were estimated using POPGENE (version 1.31) (Yeh *et al*., 1997) software. However UPGMA does not necessarily reveal a true lineage but it was appropriate to group a genetically similar prawn. Mantel's test was performed to evaluate correlation between genetic distances and geographical distances (Manly, 19930) using TFPGA version 1.3 (Miller, 1997).

Marker system comparison: RAPD marker system developed for *M. rosenbergii* was compared for information type and amount and efficiency for major research applications with LP-RAPD and RAMs marker system previously described by Bhassu *et al*. (2007). Only markers that were polymorphic across the 11 populations were included for each set of data.

RESULTS AND DISCUSSION

RAPD profile: A total of 260 prawn samples were typed using the five RAPD primers and had generated 205 markers. Scoring was done on bands generated by each primer within the molecular weight of 250 to 1500 bp as shown in Fig. 1. Generally, the percentage of polymorphic markers detected in all the primers was quite large in all the populations, which is larger than 50% (Table 2). The populations Sg. Kelantan, Sg. Linggi (Negeri Sembilan), Sg. Pahang and Sg. Penarik (Terengganu) showed the largest (100.0) and Sg. Muda (Kedah) showed the smallest percentage of polymorphism. The problem of mixed-intensity bands exacerbates the well know sensitivity of PCR to the reaction parameters. Thus, optimization of the protocol is necessary in order to obtain reproducible and interpretable RAPD banding pattern. Scoring of the banding pattern was done within the range of 250 to 1500 bp due to this range showing good reproducibility and any amplified products falls out of this range showed low reproducibility (Ambak *et al*., 2006).

Genetic distance and dendrogram: Sg. Permatang (Sabah) and Sg. Muda showed the largest genetic distance value of 0.0653 while the smallest genetic distance value was between Sg. Pahang and Sg. Penarik (Terengganu). The UPGMA dendrogram among populations is presented in

Table 2: Overall polymorphism of each population

Population	KLT	LNG	PHG	TRG	SDL	SBH	SRW	PRK	KDH	END	MR
Overall number of markers	42	51	43	43	25.0	39.0	24.0	35.0	33.0	24.0	39.0
Overall number of polymorphic markers	42	51	43	43	26.0	41.0	25.0	37.0	35.0	25.0	41.0
Overall % of polymorphism	100	100	100	100	96.2	95.1	96.0	94.6	94.3	96.0	95.1

MR: Muar, PRK: Perak, SDL: Sedili, KDH: Kedah, SBH: Sabah, SRW: Sarawak, LNG: Linggi, KLT: Kelantan, PHG: Pahang, TRG: Terengganu, ER: Endau Rompin

Table 3: Matrix of genetic distance and geographical distance

Population	KLT	LNG	PHG	TRG	SDL	SBH	SRW	PRK	KDH	END	MR
KLT	0.0000	564.00	440	168	660	2674	2274	341	351	568	652
LNG	0.0130	0.0000	231	503	369	2290	1890	345	564	321	139
PHG	0.0327	0.0287	0.0000	272	345	2383	1983	401	550	568	281
TRG	0.0317	0.0276	0.0129**	0.0000	492	2655	2255	453	463	401	544
SDL	0.0486	0.0405	0.0188	0.0142	0.0000	2583	2185	638	787	92	197
SBH	0.0384	0.0409	0.0296	0.0310	0.0492	0.0000	2000	2455	2604	2553	2389
SRW	0.0423	0.0371	0.0438	0.0389	0.0518	0.0569	0.0000	2025	2204	2153	1989
PRK	0.0398	0.0373	0.0375	0.0317	0.0439	0.0449	0.0234	0.0000	149	608	444
KDH	0.0469	0.0483	0.0487	0.0454	0.0550	0.0653*	0.0328	0.0369	0.0000	757	493
END	0.0439	0.0400	0.0435	0.0439	0.0542	0.0575	0.0276	0.0200	0.0262	0.0000	210
MR	0.0392	0.0388	0.0448	0.0371	0.0537	0.0573	0.0353	0.0374	0.0406	0.0492	0.0000

Above diagnonal is geographical distance (km), below diagonal is genetic distance (Nei, 1978),*: Highest distance, **: Lowest distance, MR: Muar, PRK: Perak, SDL: Sedili, KDH: Kedah, SBH: Sabah, SRW: Sarawak, LNG: Linggi, KLT: Kelantan, PHG: Pahang, TRG: Terengganu, ER: Endau Rompin

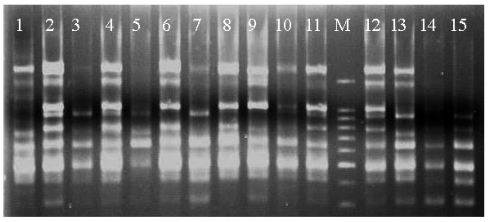

Fig. 1: RAPD patterns obtained from *M. rosenbergii* population using OPA04

Fig. 2. The dendrogram divided the populations into three major clusters. The first cluster was composed of the populations Sg. Kelantan and Sg. Linggi (Negeri Sembilan), the populations, Sg. Serian (Sarawak), Sg. Perak, Sg. Endau (Endau Rompin), Sg. Muda (Kedah) and Sg. Muar (Johor) formed the second group, where the remaining populations formed the third group. The majority of the populations did not cluster according to their geographical location, which may be caused by natural selection and mutation in order to adapt to new environments. Mantel's test showed nonsignificant correlation between genetic and geographical distance ($r = 0.0009$; $Z = 2230.3021$; upper tail $P = 0.0530$; lower tail $p = 0.9480$) (Table 3). The UPGMA dendrogram clustering showed the genetic relationships between populations not according to their geographical distribution. This situation is quite often especially for the species with large distribution area (Irwin, 2001; Qiu *et al*., 2004). This could have resulted that Pleistocene drainage basins that linked sites on the Sunda Shelf that are today geographically isolated, may have acted as conduits for gene flow among subpopulations of *M. rosenbergii*. (deBruyn *et al*., 2004). This ancient drainage basins have played a major role in shaping the distribution of molecular variation among subpopulations, thus creating the close relationship among subpopulations in Southern Thai-Malaysia and Malacca Straits River System (de Bruyn *et al*., 2005).

Data comparison: Generally, there was no significant similarity between the dendrograms constructed from UPGMA cluster analysis using data obtained by RAPD marker systems and pooled data of LP-RAPD and RAMs marker systems (Fig. 2, 3). The total number of band generated from RAPD markers was higher than the total number of band generated from LP-RAPD and RAMs markers (Bhassu *et al*., 2007). Sg. Kelantan, Sg. Linggi (Negeri Sembilan), Sg. Pahang and Sg. Penarik (Terengganu) obtained 100% of polymorphic bands for all the primers indicating these populations had high genetic variation within and between the populations. In addition, other populations also showed high polymorphism percentages, which suggested that RAPD marker is powerful approaches for the assessment of genetic variation among the freshwater prawn populations (Bhassu *et al*., 2007).

Higher number of band generated through RAPD marker system suggested that RAPD is a useful tool to assess the genetic variability while LP-RAPD and RAMs could produce more reliable and reproducible bands because of the higher annealing temperature and longer sequence of primers (Gillings and Holley, 1997; Tsumura *et al*., 1996; Nagaoka and Ogihara, 1997; Qian *et al*., 2001).

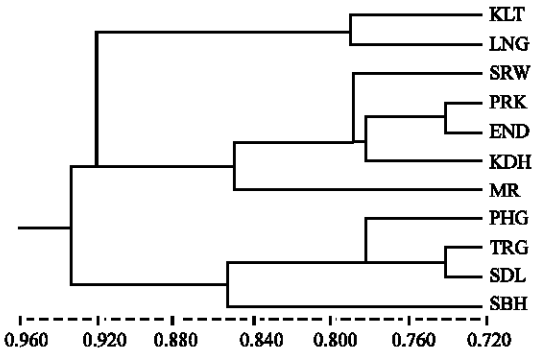

Fig. 2: UPGMA dendrogram of Malaysian giant freshwater prawn, *M. rosenbergii* based on values of genetic distance calculated from data generated for all the 5 primers, MR: Muar, PRK: Perak, SDL: Sedili, KDH: Kedah, SBH: Sabah, SRW: Sarawak, LNG: Linggi, KLT: Kelantan, PHG: Pahang, TRG: Terengganu, ER: Endau Rompin

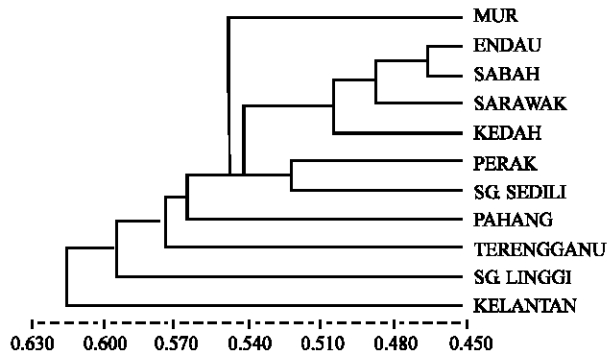

Fig. 3: Genetic relationships amongst 11 populations as revealed by LP-RAPD and RAMs markers (Bhassu *et al*., 2007)

CONCLUSIONS

RAPD fingerprinting is to yield reliable and useful results. Therefore, the risk of misinterpretation in a genetic analysis different RAPD patterns have similar size can be minimized by the use of several RAPD primers so that the genetic analysis are based on a large number of pooled RAPD markers (Bidochka *et al*., 1994). RAPD marker had been applied in studies at the individual level as well as in genetic identification and in the studies involving closely related species. Due to their very high genomic abundance, they have also been applied in gene mapping studies. Several advantages in genetic mapping providing by RAPD markers are a universal set of primers, which can be used for genomic analysis in a wide variety of species, no preliminary work such as isolation of cloned DNA probes, preparation of filters for hybridizations or nucleotide sequencing is needed and each marker is the equivalent of a Sequence Tagged Site, which can greatly simplify information transfer in collaborative research programs (Williams *et al*., 1990). In addition, this method is easy and quick to assay, requires low quantities of template DNA and no sequence data for primer construction.

ACKNOWLEDGMENTS

We would like to thank the Director of Research, Department of Fisheries, Malaysia, Mr. Ismail Awang Kechik and Mr. Chuah Hean Peng for financial support: 21-22501-006 and constant encouragement throughout the research project.

REFERENCES

Ambak, M.A., B.A.M. Ambok, P. Ismail and M.T. Bui, 2006. Genetic variation of snakehead fish (*Channa striata*) populations using random amplified polymorphic DNA. Biotechnology, 5 (1): 104-110.

Armstrong, A., A. Gibbs, R. Peakell and G. Weiler, 1996. RAPD Distance Program, Version 1.04, Australian National University, Canberra.

Bhassu, S., R. Hassan, M.Y. Chew, L.M. See, Z. Jamari and S.G. Tan, 2007. Assessing genetic relatedness among eleven populations of Malaysian giant freshwater prawn, *Macrobrachium rosenbergii* using LP-RAPD and RAMs markers. MFJ., 6 (1): 26-37.

Bidochka, M.J., M.A. McDonald, R.J. St Leger and D.W. Robert, 1994. Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA (RAPD). Curr. Genet., 25 (1): 107-113.

de Bruyn, M., J.A. Wilson and P.B. Mather, 2004. Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant freshwater prawn, *Macrobrachium rosenbergii*. Mol. Phylogenet. Evol., 30 (1): 251-257.

de Bruyn, M., E. Nugroho, M.M. Hossain, J.C. Wilson and P.B. Mather, 2005. Phylogeographic evidence for the existence of an ancient biogeographic barrier: The Isthmus of Kra Seaway. Heredity, 94 (1): 370-378.

Esselman, E.J., L. Jianqiang, D.J. Crawford, J.L. Winduss and A.D. Wolfe, 1999. Clonal diversity in the rare *Calamagrostis proteri* spp. *Insperata* (Poaceac): Comparative results for allozymes and Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers. Mol. Ecol., 8: 443-451.

Fang, D.Q. and M.L. Roose, 1997. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. Theor. Applied Genet., 95: 408-417.

FAO, 2002. Fishery statistics-Aquaculture production 2000. In FAO Year Book. FAO, Rome, Italy, pp: 180.

Gillings, M. and M. Holley, 1997. Amplification of anonymous DNA fragments using pairs of long primers generate reproducible DNA fingerprints that are sensitive to genetic variation. Electrophoresis, 18: 1512-1518.

Horn, P.L., J.A. Rafalski and P.J. Whitehead, 1996. Molecular genetic (RAPD) analysis of breeding Magpie Geese. The Auk, 113 (3): 552-557.

Irwin, R.E., 2001. Field and allozyme studies investigating optimal mating success in two sympatric spring-ephemeral plants *Trillium* and *T. grandiflorum*, Heredity, 87: 178-189.

Lynch, M., 1990. The similarity index and DNA fingerprinting. Mol. Biol. Evol., 7: 478-484.

Mather, P.B. and M. de Bruyn, 2003. Genetic diversity in wild stocks of the giant freshwater prawn (*Macrobrachium rosenbergii*): Implications for aquaculture and conservation. NAGA, WorldFish Center Q., 26: 4.

Miller, M.P., 1997. Tools for population genetic analysis (TPFGA) version 1.3. A Windows program for analysis of allozyme and molecular population genetic data. Computer software distributed by the author.

Nagaoka, T. and Y. Ogihara, 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Applied Genet., 94: 597-602.

Nei, M., P.A. Fuerst and R. Chakarborty, 1978. Subunit molecular weight and genetics variability of proteins in natural population. Proc. Natl. Acad. Sci. US., 75: 3359-3362.

Qian, W., S. Ge and D.Y. Hong, 2001. Genetic variation within and among populations of a wild rice *Oryza granulate* from China detected by RAPD and ISSR markers. Theor. Applied Genet., 102: 440-449.

Qiu, Y.X., D.Y. Hong, C.X. Fu and M.C. Kenneth, 2004. Genetic variation in the endangered and endemic species *Changium smyrnioides* (Apiaceae). Biochem. Syst. Ecol., 32: 583-596.

Rohlf, F.J., 1993. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Version 1.60. Exeter Software, New York.

Tsumura, Y., K. Ohba and S.H. Strauss, 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theor. Applied Genet., 92: 40-45.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Yang, X. and C. Quiros, 1993. Identification and classification of celery cultivars with RAPD markers. Theor. Applied Genet., 83: 2-3.

Yeh, C., R.C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao, 1997. POPGENE, the User-Friendly Shareware for Population Genetic Analysis, Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.