Indonesian Journal of Biotechnology, June, 2007 Vol. 12, No. 1, pp. 967-972

Genetic Heterogenity Profile of *Penaeus monodon* Broodstock F1 Revealed by Mitochondria DNA-RFLP and RAPD

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Abstract

The genetic heterogeneity of *Penaeus monodon* broodstock F1 was evaluated using *Restriction Fragment Length Polymorphism* (RFLP-mtDNA) and *Random Amplified Polymorphic* DNA (RAPD) analysis. The RFLP analysis was conducted by amplifying 16SrDNA region and digested with restriction enzyme *Nde* II. According to the RFLP analysis, heterogeneity value of *P. monodon* F1 broodstock population is 0,0422; male F1 population is 0,0613 and female F1 population is 0,1252. The primer OPA2 was used in RAPD analysis. According to the RAPD analysis, heterogeneity value of *P. monodon* F1 broodstock population is 0,0417; male F1 population is 0,0653 and female F1 population is 0,1104. The results of this research showed that either RFLP or RAPD can be used as a family specific marker for *Penaeus monodon*.

Key words : *Penaeus monodon*, RFLP, RAPD, heterogeneity, genetic marker

Introduction

Aquaculture has developed rapidly over the last few decades to the point where aquaculture products make up a significant proportion of the world's seafood. By far the greatest aquaculture production in terms of tonnage is oysters, and in terms of economic value is shrimp and fish rank second with fairly wide difference. However, despite this growth, domestication of aquaculture species is still in its early stage and genetic improvement program is applied only to a few species.

The knowledge of genetics of aquatic species is also limited, but meanwhile, there has been growing interest in potential value of the application of genetics to aquaculture.

Analysis of the geographical distribution of electrophoretically detectableisozyme variants has been used for some time to describe the genetic structure of natural populations and hatchery populations but the level of allozyme variation was low due to the low sensitivity of these methods (Hedgecock, *et al*., 1982; Benzie, 1998). The similar obstacle is also encountered on researches using mitochondria DNA marker to observe genetic structure of shrimp over large geographical areas (Benzie *et al*., 1993). The first information about the possibility of deleterious effect of uncontrolled inbreeding is obtained from the results of research done by Sbordoni *et al*. (1986), which indicates close correlation between a decline in spawn productivity with reduced genetic variability of allozyme variants in *P. japonicus* farmed in Italy. The second information is obtained from research conducted by Malecha and Hedgecock (1989) who attempted to maintain larger effective population sizes in breeding programs to keep the genetic variability of their offspring.

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Several genetic markers can be used to analyse genetic variation in the black tiger shrimp (*Penaeus monodon*) population. Among these genetic markers, *Restriction Fragment Length Polymorphism* (RFLP) and *Random Amplified Polymorphic DNA* (RAPD) are the most usable markers, this is because polymorphic level revealed by these analysis can show higher resolution. The information obtained from these genetic markers can provide an explanation about the relationship between genetic heterogeneity and shrimp broodstock performance that will affects the animal adaptation ability in the population. These genetic markers are used with all traditional breeding program that are based on phenotype approach. Considering the importance of this genetic research to the shrimp farming industry then some measurement need to be taken to incorporate and integrate much bigger resources, to help solve problems in the field like limited number of wild broodstock, research funding, and limited number of human resources. A collaborative effort to pool limited resources, and to provide a clear focus to attain specific goals on a timely basis, will be required to enable faster genetic improvement of valuable shrimp species. The aim of this study was to evaluate genetic heterogeneity of *Penaeus monodon* broodstock F1 produced from hatchery revealed by mtDNA-RFLP and RAPD analysis.

Materials and Method

Samples and Primers

P. monodon broodstock F1 specimen (from NSBC division, MCBAD Jepara), 10% Chelex-100 (Bio-Rad), Proteinase K (20 mg/l; Roche), restriction enzyme: *Nde* II (/GATC; Roche), RFLP's primers : 16SrDNA (5'-CGC CTG TTT AAC AAA AAC AT-3' dan 5'-CCG GTC TGA ACT CAG ATC ATG T-3'; Sigma), and RAPD's primer : OPA 2 (5'-TGC CGA GCT G-3'; Sigma), PCR kit reagensia (Roche), electrophoresis gel, 1x buffer TBE, DNA ladder 100 bp, ethidium bromide

Equipment

Mini centrifuge, thermoblock, PCR Rack 96 well, cryobox 9 X 9 array, pipette stand, mikropipet, PCR thermocycler, electrophoresis equipments, water bath, UV transluminator.

Sampling

Twenty seven individuals of *P. monodon* F1 (18 male and 9 female individuals) were captured alive from broodstock pond NSBC BBPBAP Jepara. The progenitor of these F1 broodstock were captured from Sunda strait. Pleopods were removed from each shrimp and immediately placed on dry ice before stored at –20°C for further use.

DNA Extraction

Genomic DNA was extracted from frozen pleopods of each individual using 10% chelex-100 and Proteinase K, followed Ovenden, (2000) procedure.

DNA Amplification

The amplification of genomic DNA was conducted either by RFLP or RAPD procedures as follows:

I. mtDNA-RFLP analysis

16S rDNA region was amplified by PCR using 16SrDNA-1 (5'-CGC CTG TTT AAC AAA AAC AT-3') and 16SrDNA-2 (5'-CCG GTC TGA ACT CAG ATC ATG T-3') primers. Polymerase chain reaction (PCR) was conducted in 25 µl reaction volume containing : 2,5 µl genome, 11 µl H_2O, 2,625 µl 10 x buffer, 2,625 µl dNTP (2,5 mM), 0,625 µl for each primers (10 mM), 5,25 µl $MgCl_2$ (25 mM), and 0,25 µl Taq (10 mM). Reactions was conducted in PCR thermocycler with the following steps : pre denaturation at 93°C for 2 min followed by 30 cycles consists of denaturation at 93°C for 30 s, annealing at 50°C for 30 s and synthesis at 72°C for 45 s. Extension at 2°C for 5 min was held at the end of cycles. PCR yields was then

stored at 4°C.

Five microliters of each amplification reaction was electrophoresed through a 1,0% agarose gel to determine whether the resulting fragment was succesfully amplified. Four microliters of these amplified fragments were then singly digested with *Nde* II under the conditions : 0,6 µl H_2O, 1,2 µl Buffer; 0,2 µl RE; and 4,0 µl PCR product, and incubated at 37°C for 3.5 hours.

II. RAPD analysis

Nuclear DNA region was amplified by PCR using OPA 2 primer (5'-TGC CGA GCT G-3'). Polymerase chain reaction (PCR) was conducted in 25 µl reaction volume containing : 1,25 µl genome; 11,75 µl H_20; 2,625 µl 10 x Buffer; 2,625 µl dNTP (2,5 mM); 1,25 µl primer (10 mM); 5,25 µl $MgCl_2$ (25 mM); and 0,25 µl Taq (10 mM). Reactions were conducted in PCR thermocycler with the following step : initial denaturation at 94°C for 4 min, followed by 35 cycles consist of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and synthesis at 72°C for 2 min. Cycles were end by final extension at 72°C for 5 min and stored at 4°C.

Data Analysis

Fragmentation pattern produced by the digestion of mitochondria segment by restriction enzyme *Nde II* is then arranged in alphabetical order based on the digestion number. These fragmentation pattern was the main data to calculate the heterogeneity level in the broodstock population, and also in the male and female population of *P. monodon* broodstock F1.

Results

Table 1 DNA fragments of *Penaeus monodon* digested by six restriction enzyme

No	Species	DNA Fragment	Restriction enzyme					
			Nde II	*Hae* III	*Hind* III	*Hinf* I	*Eco* RV	*Bam* HI
1	*P. monodon*	125	-					
		175	-					
		300	-					
		400	-					
		425	-					
		500		-	-	-	-	-

Figure 1. Fragmentation pattern of *P. monodon* broodstock F1 mitochondria DNA using RFLP marker on *Nde* II locus. (note : lane 1 : Marker, lane 2-17 : fragmentation pattern of mitochondria DNA).

Table 1 show the DNA fragments of *Penaeus monodon* digested by six restriction enzyme (*Nde* II, *Hae* III, *Hind* III, *Hinf* I, *Eco* RV and *Bam* HI), but only one of them that is *Nde* II which showed the digestion on the mitochondria DNA with the DNA length of 125, 175, 300, 400 dan 425 bp (Figure 1).

Table 2. DNA Fragment (bp) of *P. monodon* broodstock F1 according to the RAPD marker.

No	Species	Primers	
		OPA 2	B 20
1	*P. monodon*	700 825 1100 1200 1400	No amplification

Table 2 show DNA fragments of *P. monodon* broodstock F1 revealed by RAPD using two primers OPA2 and B20, but only OPA2 primer can show RAPD fragments in 700, 825, 1100, 1200 and 1400 bp (Figure 2).

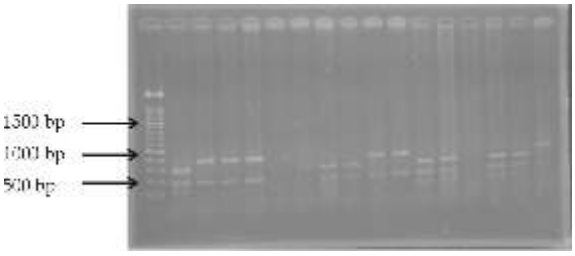

Figure 2. Fragmentation pattern of *P. monodon* nuclear DNA using RAPD marker. (note : lane 1 : Marker, lane 2-8 : fragmentation pattern of nuclear

DNA)*Fragmentation pattern and heterogeneity value*

Either RFLP or RAPD analysis of *P. monodon* broodstock F1 revealed 3 fragmentation patterns (Table 3).

Table 3. Restriction fragment pattern (RFLP) and fragmentation pattern (RAPD) of *P. monodon* broodstrock F1.

Genetic Marker	Species	Primer / Restriction Enzim	Fragmentation pattern
RFLP	*P. monodon*	16SrDNA *Nde* II	C : 125, 175, 400 D : 125, 175, 300, 400 E : 125, 175, 400, 425
RAPD	*P. monodon*	OPA2	C : 700, 825, 1200 D : 700, 825, 1100, 1200 E : 700, 825, 1100, 1200, 1400

Based on these fragmentation patterns, the heterogeneity value then can be calculated. The heterogeneity value comprised of value in the population, male population dan female population from *P. monodon* broodstock F1 (Table 4).

Tabel 4. Heterogeneity value of *P. monodon* F1 population, male and female population revealed by RFLP and RAPD analysis.

No	Species	Marker	Heterogeneity value		
			Population	Male	Female
	P. monodon	RFLP	0,0422	0,0417	0,1252
2	*P. monodon*	RAPD	0,0417	0,0422	0,1139

Table 4 show that there are no significant differences in heterogeneity value of *P. monodon* broodstock F1 population, male and female population between RFLP and RAPD analysis. It is indicated that the heterogenity value of male population is quite lower compared to female population according these two analysis.

Discussion

The results of this research has shown that either RFLP or RAPD was potential to be used as family specific marker for *P. monodon* genetic analysis. The major strength of RFLP marker is that they are codominant marker, i.e., both alleles in an individual are observed in the analysis. Be cause the difference in size is often large, scoring is relatively easy. The major disadvantage of RFLP is the relatively low level of polymorphism. RAPD marker itself is relatively new marker analysis compared to RFLP. One of the major strengths of RAPD marker is that they required short primers (8-10 bp) and relatively low annealing temperatures (often 36-40°C). Therefore, the likelihood of amplifying multiple products is great, with each product (presumably) representing a different locus. RAPDs have all the advantages of a PCR-based marker. Beside, they have another benefit that is primers are commercially available and do not require prior knowledge of the target DNA sequence or gene organization. The major disadvantage of RAPD is that they are subject to low reproducibility due to the low annealing temperature used in PCR amplification. These difficulties have limited the application of this marker in fishery science (Wirgin and Waldman, 1994). Eventhough these two analyzis have a different criteria, the results indicated that there are significant differences in their analysis. The value of heterogeneity from the *P. monodon* broodstock F1 population show almost similar results that is 0,0422 and 0,0417. Similar results also appear in the heterogeneity value of male and female population.

Shrimp farming industry has grown in South East Asia, but two fundamental problems have risen leading to great economic loss. The first problem is caused by viral diseases that affect shrimp health resulting in large economic losses in affected countries. The second problem is concerned with the effects of domestication on genetic diversity levels in cultured lines. Genetic diversity is likely to have been severely

impacted during the domestication process, besides, as a result of culture stocks, they were exposed to repeated bottlenecks as they were developed in culture. This has led to an increasing in consanguinity over time. Full sib and half sib production in hatcheries can also impact on genetic diversity levels potentially compromise the productivity of lines that will be obtained in successive generations. Over time of this process can deplete reproductive parameters and larval survival, increase malformation rates and increase susceptibility to diseases. The decrease in genetic heterogeneity is indicated in these results, heterogeneity value as much as 0,0417-0,0422 is quite a low value compared to the value of wild population and the other broodstock region. It can be assumed that Sunda strait has a low genetic potency for their *P. monodon* broodstock compared to other region (Aceh and Timika) and also the relatively low number of broodstock used in the hatchery makes the genetic heterogeneity of the broodstock very low. The disadvantages of using these low genetic heterogeneity broodstock has been mentioned before, but unfortunately, these disaadvantagesnot only affect the broodstock themselves but also can be harmful to native populations. It may lead to a reduction in the effective population size (EPS), even if no exogenous genetic material is introduced and the released fish are not adapted to the artificial rearing conditions. This will compromise the genetic diversity in receiving stocks. Although the introduction of genetic material in wild population may be in some instances advisable, for example when a natural stock has been depleted for a long time, in most cases this practice has potentially negative consequences for the genetic integrity of receiving populations. The inundation of a broad area by introduced genetic material may affect the genetic structure and variation levels in wild stocks and lead to genetic homogenization of populations in the area. Valuable gene pools or co-adapted gene complexes can also be lost through replacement by exogenous genes, a phenomenon known as genetic introgression. Since the existence of natural population subdivision may imply adaptation to local conditions, genetic assessment of the degree of population structuring and gene flow among natural populations are practical ways of helping to preserve existing biodiversity and maintaining valuable adaptive populations, as well as for monitoring changes in the genetic composition of receiving populations after release. This approach should constitute an integral part of any translocation or restocking program, especially for penaeid species.

The calculation of heterogenity value between male and female population of this F1 broodstock shows an interesting result where the heterogeneity value of male population is quite low (0,0613) compared to female population (01252). The reason for the difference in value between male and female population is not clear, but it can be assumed due to random genetic drift phenomenon or because of the founder effect. In the future, presumably we can use the Sunda strait female broodstock population as a F1 hybrid. It needs more research to gain detail information about the difference genetic heterogeneity between male and female population of *P. monodon* broodstock in the wild and their cohort generation.

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