Full Paper

BIOCHEMICAL GENETIC ANALYSIS OF THREE POPULATION OF MARBLE GROUPER, *Epinephelus polypekhadion*

ANALISIS GENETIK BIOKIMIAWI DARI TIGA POPULASI IKAN KERAPU BATIK, *Epinephelus polypekhadion*

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Abstract

Genetic variations of marble groupers, *Epinephelus polypekhadion* were evaluated to know genetic performance of fish to support fish seed production. Fifty fish samples from three locations i.e. West Nusa Tenggara, South Sulawesi and East Java were collected for analysis. Genetic analysis has been examined through allozyme electrophoresis by using 11 enzymes (ADH, GPI, SDH, IDH, LDH, ME, PGM, 6PGD, MPI, SP, EST). The result showed that 16 loci were detected, and three of them were polymorphism enzymes namely Isocitric dehydrogenase (IDH*), Glucose Phosphate Isomerase (GPI-1*) and Esterase (EST-2*). One locus (EST-2) was polymorphic in the East Java and West Nusa Tenggara populations and three loci (EST-2, GPI-1 and IDH) were polymorphic in the South Sulawesi population. The heterozygosity ranged from 0.007 to 0.034. Rogers genetic distance between population pairs were ranged from 0.005 to 0.012 (average = 0.009). Differences between genetic populations were significant (P<0.05). East Java and West Nusa Tenggara populations have same gene pool.

Key words: allozyme electrophoretic, genetic variation, marble grouper

Introduction

Sixty three species of genus *Epinephelus* have been reported by Randall (1987). At least 21 of them have commercial culture value and source of protein (Shamsudin, 1992). The marble grouper, *Epinephelus polyphekhadion* is widely distributed throughout the tropical and sub tropical Indo-West Pacific region, Red Sea and from East Coast of Africa to French Polynesia. Due to high economic value, this species is promoted to be cultured in Indonesia waters. Recently, aquaculture industries in Singapore and Indonesia have been interested to culture this species. The demand of grouper is rapidly increase in Asia and Pacific, mainly Singapore, Hongkong, Taiwan and Southern China (Rimmer *et al.,* 2004). The grouper hatcheries has been successfully developed.

There is little information available on the molecular genetics of this species. One of most advances method to estimate genetic similarity of populations is based on protein electrophoresis (Ayala, 1975). Many biochemical genetic markers have become available through the electrophoresis technique, which has revealed a large amount of molecular variation in proteins and enzymes. This paper describes electrophoretic investigation levels of genetic variability among different populations of the marble

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grouper. The results are important for future development of grouper breeding and management wild populations.

Materials and Methods

Samples collection

A total of 150 wild fish E. polypekhadion of 33.55 to 36.10 cm in total length range were caught from three locations in Pangkep (South Sulawesi), Lamongan (East Java) and Sumbawa (West Nusa Tenggara) (Table 1).

Biochemical techniques

Sample preparation

Samples (muscle, liver, heart and eye) for electrophoresis analysis were prepared through mechanical disruption, if the samples are not immediately analyzed, the tissue was stored in liquid nitrogen or in -40°C freezer.

Preparation of gels and buffer

Starch gels were made by boiling a mixture of 12.5% hydrolysed starch potatoes (Sigma-S.4501) and suitable buffer, tris citric acid pH-8 (TC-8) or citric acid aminoprophylmorpholine pH-6 (C-APM-6). The gels (20x13x1 cm) were cooled with ice pack during the electrophoresis. According to Whitemore (1990) for each tissue, several electrophoretic buffers should be tried, in order to determine which buffer produces best detection (intense, well separate, sharp and clear of band).

Electrophoresis

Small filter paper (blotting paper) were dipped into the tissue (muscle, liver, heart and eye) suspension, blotted on paper towell and inserted into a slit of gel. Allozyme electrophoresis is carried out at room temperature.Electric current is 40 mA/plate with CAPM-6 and TC-8 buffer. A small amount of bromophenol blue is inserted into various part or some wells as marker. This marker migrated slightly faster than protein and useful as indicator of migration.

Enzymatic staining

Enzyme analysis was done using horizontal gel electrophoresis recipes (Sugama & Prijono, 1998;Taniguchi & Sugama, 1990). Enzymes staining recipes followed Shaw & Prasad (1970). Enzymes which examined were: alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), isocitrate dehydrogenase (IDH), malic enzyme (ME), phosphoglucomutase (PGM), glucose 6 phosphate isomerase (GPI), 6-posphoguconate dehydrogenase (6-PGD), esterase (EST) and aspartate aminotransferase (AAT).

Data analysis

Deviations from expected Hardy Weinberg genotype proportions were tested by χ^2. The genetic variation among population were tested using the software package GENEPOP (Raymond & Rousset, 1995). Loci and allele were named according to the conventions described in Allendorf & Utter (1979). Genetic distance and differentiation among samples were quantified by Rogers (1972) formula and the dendogram was constructed from the matrix genetic distance using un-weighted pair group method with arithmetic average (UPGMA) (Sneath & Sokal, 1973).

Table 1. Means of body length and body weight of marble grouper, *E. polypekhadion* from different location

No.	Locations	Date	Total length (cm)	Weight (g)	No. of samples
1.	West Nusa Tenggara	17-11-2000	36.10±9.98	1015.2±937	50
2.	South Sulawesi	25-05-2000	33.55±6.19	684.2±310	50
3.	East Java	20-08-2000	34.94±3.87	763.0±251,5	50

Result and Discussion

Tissue and buffer specific

The activity of enzymes were highest in muscle and some in liver. The analyzed enzymes, presumptive loci, tissue sources, and buffer systems are shown in Table 2.

Genetic variation

Among eleven enzymes which examined, sixteen loci were monomorphic in all three populations. Three loci, Idh* (isocitrate dehydrogenase), Gpi-1* (glucose 6 phosphate isomerase) and Est-2* (esterase) were polymorphic as showed in Fig.1.

Table 2. Specific tissue and buffer systems for allozyme electrophoresis of marble grouper, *E. polypekhadion*

No.	Enzymes (E.C. number)	Locus	Tissue	Buffer	Polymorphism
1	Alcohol dehydrogenase (1.1.1.1)	Adh*	L	C-APM-6	M1
2	Aspartate aminotransferase (2.6.1.1)	Aat-1*	M	C-APM-6	M1
		Aat-2*	L,M	C-APM-6	M1
3	Esterase (3.1.1.3)	Est-1*	L	C -APM-6	M1
		Est-2*	L	C-APM-6	P
4	Glucose phosphate isomerase (5.3.1.9)	Gpi-1*	L,H	C-APM-6	M1
		Gpi-2*	L	C-APM-6	P
		Gpi-3*	M	C-APM-6	M1
5	Isocitrate dehydrogenase (1.1.1.42)	Idh-1*	L	C-APM-6	P
6	Lactate dehydrogenase (1.1.1.27)	Ldh-1*	M,H	C-APM-6	M1
7	Malate dehydrogenase (1.1.1.37)	Mdh*	M	C-APM-6	M1
8	Malic Enzyme (1.1.1.40)	Me-1*	L,H	TC-8	M1
		Me-2*		TC-8	M1
9	Phosphoglucomutase (2.7.5.1)	Pgm*	L,M	C-APM-6	M1
10	6-Phosphogluconate dehydrogenase (1.1.1.44)	6-Pgd*	L	C-APM-6	M1
11	Sorbitol dehydrogenase (1.1.1.14)	Sdh*	L,H	TC-8	M1

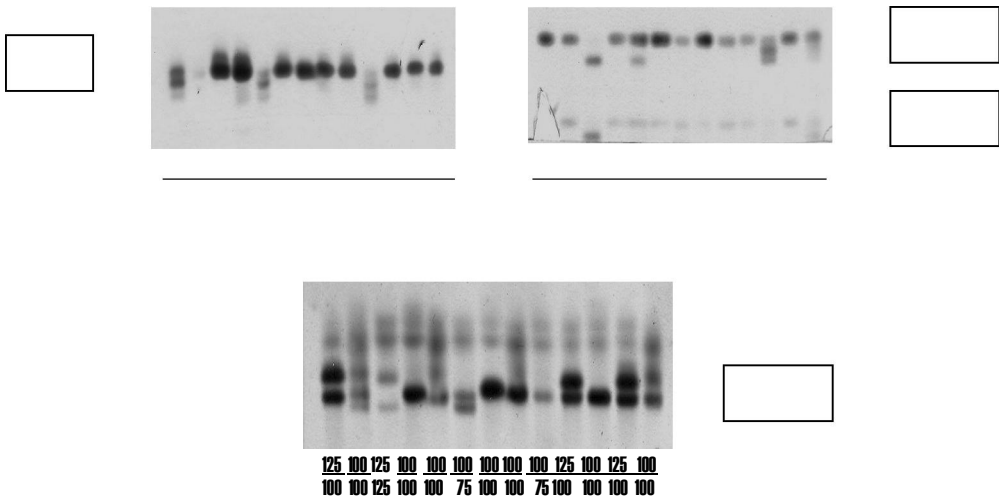

Figure 1. Zymogram polymorphic loci of marble grouper *E. polypekhadion*

The protein structure of Isocitrate dehydrogenase (IDH) and glucose 6 phosphate isomerase GPI *100, GPI*90 enzyme were dimer and controlled by two allele IDH*100, IDH*90. Locus 2 of esterase enzyme controlled by three allele Est*75, Est*100, and Est*125. Allele frequency of locus polymorphism was shown in Table 3. G-statistic (Sokal & Rohlf, 1981) for allele frequency homogenity was significantly different for three polymorphic loci ($P< 0.01$).

The same dominant allele at each locus was common in each populations surveyed. Genetic variation was consistently low in all populations (Table 4). The proportion of polymorphic loci ranged from 0.060 to 0.180 (average : 0.10) and the number of allele per locus ranged from 1.12 to 1.25 (average : 1.16). Average of observed heterozygosity was 0.019. Ratio Ho/He population of South Sulawesi more than 1 indicated that genetic variation higher than other population. If compared to milkfish from natural population which having locus polymorphic proportion of 48%, amount of allele per locus (1.52) and heterozygosity (0.045) (Permana *et al*., 2001a), and humpback grouper (Ho : 0.089) (Permana *et al*., 2001b), marble grouper had very low genetic variation (average : 0.019).

Table 3. Allele frequencies and Hardy-Weinberg expectation at polymorphic loci of marble grouper, *E. polypekhadion.*

Enzymes	Allele	Allele frequency		
		West Nusa Tenggara	South Sulawesi	East Java
Gpi-1*	B	1.000	0.930	1,000
	C	0.000	0.070	0.000
	χ^2	0.000	0.000	0.000
Idh*	B	1.000	0.970	1,000
	C	0.000	0.030	0.000
	χ^2	0.000	0.000	0.000
Est-2*	A	0.158	0.122	0.097
	B	0.842	0.847	0.806
	C	0.000	0.031	0.097
	χ^2	3.03	0.000	0.000

Table 4. Summary of genetic variability of marble grouper, *E. polypekhadion* heterozygosities (Ho) and number of allele per loci (Na) at polymorphic loci based on 16 loci.

Item	West Nusa Tenggara	South Sulawesi	East Java
Number of sample examined	50	50	50
Number of loci examined	16	16	16
Number of polymorphic loci	1	3	1
Proportion of polymorphic loci	0.06	0.18	0.06
Number of allele per locus	1.12	1.25	1.12
Heterozygosity:			
Observed (Ho)	0.007	0.034	0.016
Expected (He)	0.017	0.033	0.021
Ho/He :	0.411	1.03	0.761

According to Grant *et al*. (1981), the difference of allele frequency of marine fish populations was resulted from the action of three forces, i.e. migration, random genetic drift and natural selection. They also suggested that little or no genetic variation was expected between populations of marine fishes due to high potential for gene flow between populations. The genetic difference of marble grouper in this study could be explained by the lack of migration or gene flow particularly between populations of South Sulawesi and the others (East Java, West Nusa Tenggara). Those locations were isolated from others by geographical distance. Consequently there was no genetic differentiation observed between East Java and West Nusa Tenggara.

The genetic difference among West Nusa Tenggara population, East Java and South Sulawesi populations is caused by habitat which an individual, poly settles is genetically determined. That is alternative genotypes preferentially settle in different habitat such as in Bali Sea and Sulawesi Strait. The genetic distance was computed according to Rogers (1972).

Genetic distance of population between West Nusa Tenggara and East Java was shorter (0.005) compare to South Sulawesi population (genetic distance value 0.0115) (Figure 3). The mean F_{st} values of three polymorphisms loci of 0.145 indicated that about 14% of the total gene diversity observed was due to population differentiation and almost 86% was due to variation among individuals within species. Based on this finding the authors propose to use the parent from South Sulawesi to minimize the lost of genetic variation in fish breeding.

Conclusions

Fish population at South Sulawesi has higher genetic variation (0.034) than population of West Nusa Tenggara (0.007) and East Java (0.016). East Java and West Nusa Tenggara populations have same gene pool with closely genetic distance (0.005) and South Sulawesi population has far genetic distance value (0.0115).

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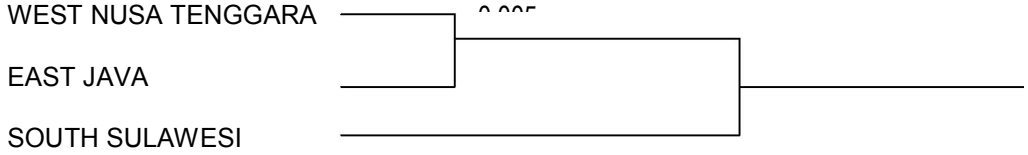

Figure 3. Genetic distance of marble grouper, *E. polypekhadion*

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