GENETIC IDENTIFICATION FOR TUNA AND RAINBOW RUNNER CAPTURE IN NORTH BALI WATERS

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ABSTRACT

Gondol Research Institute for Mariculture identification of tuna and rainbow runner was an objective in this current study. Samples of five species were collected from territorial water of North Bali. The method used in this study was allozyme electrophoresis. The results showed that buffer of CAPM-6 (*citric acid aminoprophylmorpholine*) resulted in a sharp and clear banding pattern. The species could be differentiated in six diagnostic isozyme patterns Idh* (*isocitrate dehydrogenase*), 6Pgd* (*6 phosphogluconate dehydrogenase*), Gpi* (*glucose phosphate isomerase*), Mdh* (*malate dehydrogenase*), Est* (*esterase*), and Sp* (*sarcoplasmic protein*). All species were in Hardy-weinberg equilibrium. Heterozygosities of species were ranged from 0.00 to 0.099. Yellowfin tuna has the highest heterozigosity compared with the other species. Clustering samples according to pairs revealed that genetic distance of Bullet tuna (*A. rochei*) and Eastern little tuna (*E. affinis*) had small value (0.001). By contrast, the largest value was observed between yellowfin tuna, *T. albacares* and rainbow runner, *E. bipunnulata* (0.007). This value indicated that Bullet tuna (*A. rochei*) and Eastern little tuna (*E. affinis*) closed relation, while among yellowfin tuna, skipjack tuna, and rainbow runner, were separated phylogenically.

KEYWORDS: tuna, genetic identification, heterosygozity

INTRODUCTION

Tuna, *Thunnus* sp. is representing type of fish having important economic value in fishery in the world. Tuna consists of *yellowfin, bigeye, bluefin, albacore*, and another species in Indonesian territorial water. Specially at territorial water of north Bali waters some fish caught consisted of yellowfin tuna, *(Thunus albacares)*, skipjack tuna *(Katsuwonus pelamis)*, eastern little tuna *(Eat hynus affinis)* bullet tuna *(A uxis rochei)* and rainbow runner *(Elgatis bipunnulata)*. One of the fifth species is Yellowfin tuna *(Thunnus albacares)* as being developed at Gondol Research Institute for Mariculture to support restocking and stock enhancement program. However, the biological data and the basic data related to genetic variation is a vital importance. Very few observations have been made on the genetic character of Tunas, Thunus sp. Generally classification is based on differentiation of morphological and color appearance. Allozyme electrophoresis analysis was applicable for identifying species (Menezes *et al.*, 1993) and also to know variation of gene in a population (Taniguchi & Sugama, 1990). This technique also widely used to detected variation of gene which implied in species (Taniguchi & Sugama, 1990). Electrophoresis technique also applicable to determine genetic relationships of species and as indicator to determine the possibility of hybrid among species (Grant & Stahl, 1988).

MATERIALS AND METHODE

Sample were obtained in coastal of North Bali waters (Figure 1). Five types of fish caught were: yellowfin tuna (*Thunnus atbacares*), skipjack tuna, (*Katsuwonus pelamis*), bullet tuna (*Auxis rochei*), eastern little tuna (*Euthynus affinis*), and rainbow runner (*Elgatis bipunnulata*). Fish were captured at depth of 800—1,000 m. Tissue samples utilized for the analysis were muscle, liver, heart, and eye.

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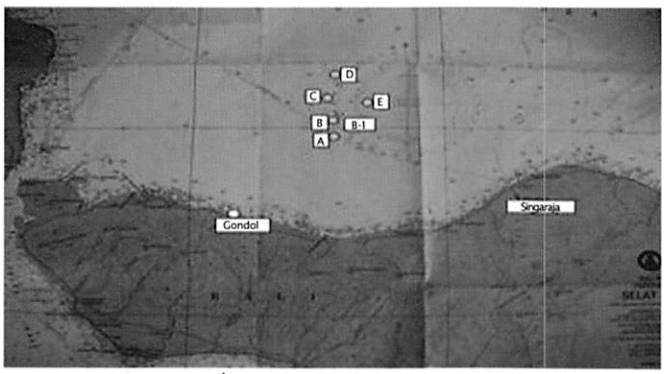

A (S 08°01.636' E 114°50.734')
B (S 07°59.472' E 114°50.331')
B-1 (S 07°58.765' E 114°51.331')
C (S 07°56.480' E 114°50.813')
D (S 07°54.130' E 114°51.887')
E (S 07°57.665' E 114°53.791')

Figure 1. Map of fishing area in territorial of North Bali waters

Allozyme Electrophoresis

Enzyme variation were analyzed using horizontal starch gel electrophoresis as following method developed by Taniguchi & Sugama (1990). Concentration of gel for electrophoresis was 12% w/v, using hydrolyzed starch (Sigma *S-4501).* Electrophoresis was conducted at 4 mA/cm² with 14 enzymes were used in this analysis. The enzyme was alcohol dehydrogenase (ADH. E.C.1.1.1.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), Ü-glycerol phosphate dehydrogenase (GPD. E.C. 1.1.1.8), lactate dehydrogenase (LDH, E.C. 1.1.1.27), sorbitol dehydrogenase (SDH, E.C.1.1.1.27), xanthine dehydrogenase (XDH, 1.1.1.7) isocitrate dehydrogenase (IDH. E.C. 1.1.1.42), 6- phosphogluconate dehydrogenase (6- PGD, 1.1.1.44), malic enzyme (ME, E.C. 1.1.1.40), glucose phosphate isomerase (GPI. E.C. 5.3.1.9), phosphoglucomutase (PGM, 2.7.5.1), mannosephosphat isomerase (MPI, E.C), esterase (EST, E.C. 3.1.1.3), and sarcoplasmic protein (SP). Conmon allele given number 100 of this matter according to Shaklee *et al.* (1990). Staining of enzyme as following method by Shaw & Prasad, (1970). Deviation from expected Hardy-Weinberg Genotype proportions were tested by Chi-square. Test for a relationship between genetic differentiations were tested in 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 was quantified by Rogers (1972), formula and the dendogram was constructed from the matrix genetic distance using Unweighted Pair Group Method with Arithmetic averages/UPGMA (Sokal & Rohlf, 1981).

RESULT AND DISCUSSION

The analyzed enzymes, presumptive loci, tissue source and buffer systems are shown in Table 1. Most enzymes were active in muscle and liver while some expression enzymes specified in heart and eye, but pattern had faint banding. Enzyme of Ldh, Me, Mdh, Gpd, and SP avtivity in muscle while other enzyme activity such as Adh, Idh, 6-Pgd, Pgm, Mpi, Gpi, Sdh, Xdh, and Est expression in the liver.

ALLOZYME MARKER

From the fourteen enzymes analyzed, six enzymes were detected as allozyme markers for tuna yellowfin tuna (*T. albacares*), skipjack tima (*K. pelamis*), bullet tuna (*A. rochei*), eastern little tuna (*E. affinis*), and rainbow runner (*E. bipunnulata*). Genetic interpretation of handing pattern of Tunas and rainbow runner is shown in Figure 2.

IDH: One locus detected for this enzyme, controlled by four alleles that are IDH*80 IDH*100, IDH*115, and IDH*120. This Enzyme activity in liver. Three species *K. pelamis*, *A. rochie* and *E. bipunnulata* were fit at allele IDH*80. *E. affinis*, were fit at IDH* 115, and IDH*120, and *T. albacares* at allele IDH* 100.

GPI: Two loci were detected for this enzyme and controlled by four alleles: GPI*90, GPI*100, GPI*110, and GPI*140. Two species (*K. pelamis* and *E. bipunnulata*) were fit at allel GPI*100, while *A. rochie* at GPI*100, GPI*110, and *T. albacares* at allele GPI*90 and *E. affinis*, at GPI*140.

6-PGD: One locus were detected for this enzyme and controlled by three alleles 6PGD*

Table 1. Tissue and specific buffer allozyme electrophoresis of tunas and rainbow runner

Enzyme (E.C. Number)	Locus	Tissue assayed	Buffer	Mobility
Alcohol dehydrogenase (1.1.1.1)	Adh*	Liver	TC-8	Anode
a-Glycerol phosphate dehydrogenase (1.1.1.8)	µGpd-1*	Muscle	C-APM-7	Cathode
	µGpd-2*	Liver		
Lactate dehydrogenase (1.1.1.27)	Ldh*	Muscle	C-APM-7	Cathode
Malate dehydrogenase (1.1.1.37)	Mdh-1*	Muscle	TC-8	Cathode
	Mdh-2*	Liver		
Isocitrate dehydrogenase (1.1.1.42)	Idh*	Liver	C-APM-7	Cathode
		Muscle		
Phosphoglucomutase (2.7.5.1)	Pgm-1*	Liver	C-APM-7	Cathode
6-Phosphogluconate dehydrogenase	6-Pgd*	Liver	C-APM-7	Cathode
Glucose phosphate isomerase (5.3.1.9)	Gpi-1*	Liver	C-APM-7	Cathode
	Gpi-2*			
Sorbitol dehydrogenase (1.1.1.14)	Sdh1*	Liver	TC-8	Cathode
	Sdh2*			Anode
Xanthine dehydrogenase (1.2.3.2)	Xdh*	Liver	TC-8	Cathode
Malic enzyme (1.1.1.40)	Me-1*	Muscle	TC-8	Cathode
	Me-2*			
Mannose phosphate isomerase (5.3.1.8)	Mpi*	Liver	TC-8	Cathode
Sarcoplasmic protein	Sp-1*	Muscle	C-APM-7	Cathode
	Sp-2 *			
Esterase (3.1.1.3)	Est*	Liver	TC-8	Cathode

100, 6PGD*90, 6PGD*60. Three species *K. pelamis, A. rochie,* and *E. bipunnulata* were fit at allele 6PGD*100 and 6PGD*90, while *T. albacares* was fit at allele 6PGD and *E. affinis* was not detected.

SP: Two loci detected at this enzyme, controlled by four alleles SP*90, SP100, SP*115, and SP*120. Three species *T. albacares, K. pelamis,* and *E. affinis* were fit and controlled by same allele SP100, and SP*120. While two species *A. roche* and *E. bipunnulata* controlled by same allel that is SP*80 and SP*115.

MDH: Two loci were detected in this enzyme controlled by five alleles MDH*85, MDH*90, MDH* 100, MDH*115, and MDH*120. *A. rochie* and *E. bipunnulata* had couple of the same allel MDH*90 and MDH* 100. While *T. albacares* was in (MDH*85 and MDH*115 *K. pelamis* was in allel (MDH*85 and MDH*100) and *E. affinis,* was in MDH*90 and MDH*120.

EST: Two loci were detected in this enzyme, controlled by five allele that are EST*80, EST*90 EST*100, EST*115, EST*120. *A. rochie* and *E. bipunnulata* had couple of the same allele (EST*100 and*120). *T. albacares* were fit at alleles EST*115, *K. pelatnis* were fit at (EST*100 and EST*120), and. *E. affinis* were fit at (EST*80 and EST*90).

GENETIC VARIATION

From 14 enzyme analyzed 20 locus were detected and three of them were polymorphic (GPI*1, IDH*, EST-*). The value of heterozygosity observed (Ho) ranged from 0.00—0.008. The proportion of polymorphic loci among five species ranged from 0.0—0.15. The number of alleles per locus ranged from 1.00—1.208. The

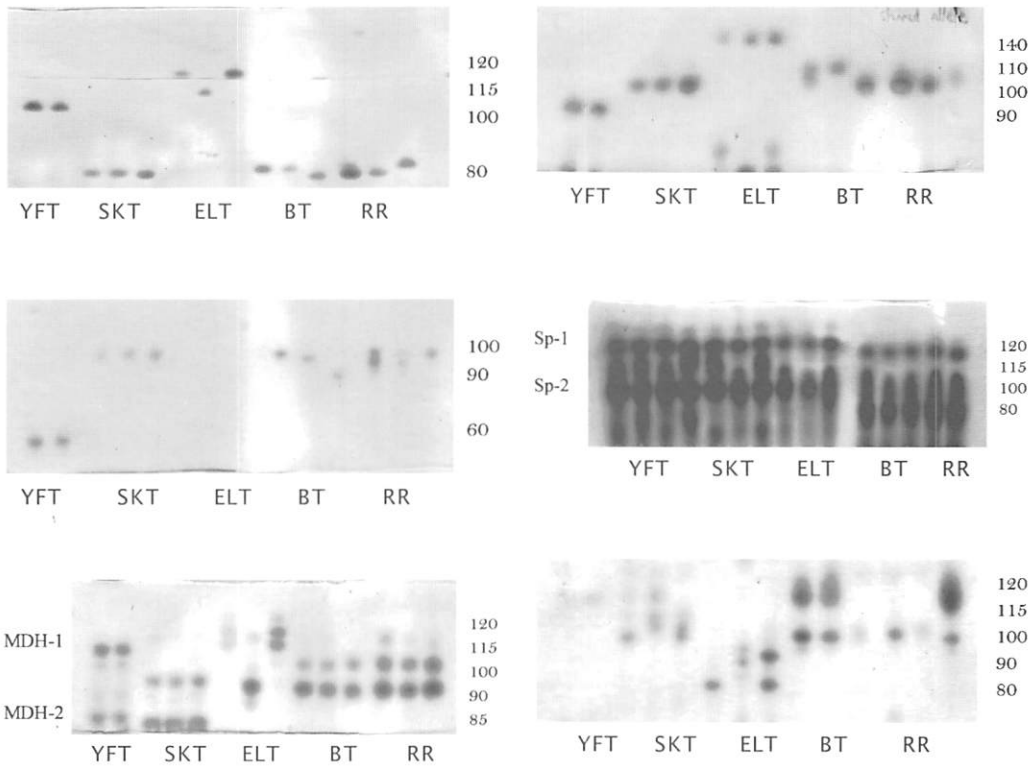

Figure 2. Genetic interpretation of banding pattern of Tunas, *Thunus* sp. of the 14 enzymes examined. YFT yelowfin tuna (*T. albacares*), SKT: skipjack tuna (*K. pelamis*), ELT: eastern little tuna (*E. ffinis*), BT: bullet tuna (*A. rochie*), RR: rainbow runner (*K. bipunnulata*)

Table 2. Summary of genetic variability, proportion of polymorphic loci, number of alleles per loci, heterozygosity of tunas and rainbow runner

Description	Yellowfin tuna	Skipjack tuna	Eastern little tuna	Bullet tuna	Rainbow runner
No of samples examined	40	20	20	20	20
No of loci examined	20	18	18	18	20
No of polymorphic loci	3	3	0	1	3
% Polymorphic loci	0.15	0.083	0	0.05	0.15
No of alleles per locus	1.208	1.125	1	1.041	1.25
Heterozygosis (Ho)	0.008 ± 0.006	0.005 ± 0.04	0.00 ± 0.0	0.001 ± 0.03	0.004 ± 0.03

value of heterozygosity of five species is shown in Table 2.

The averages of heterozygosity were ranged from 0.00—0.008. Yellowfin tuna (*T. albacares*) had the highest heterozygosity value (0.008). The smallest value of heterozygosity was observed in eastern little tuna (0.00) and the largest value was observed in yellowfin tuna (0.008), (Figure 3). it indicated yellowfin tuna had high variation to control (growth

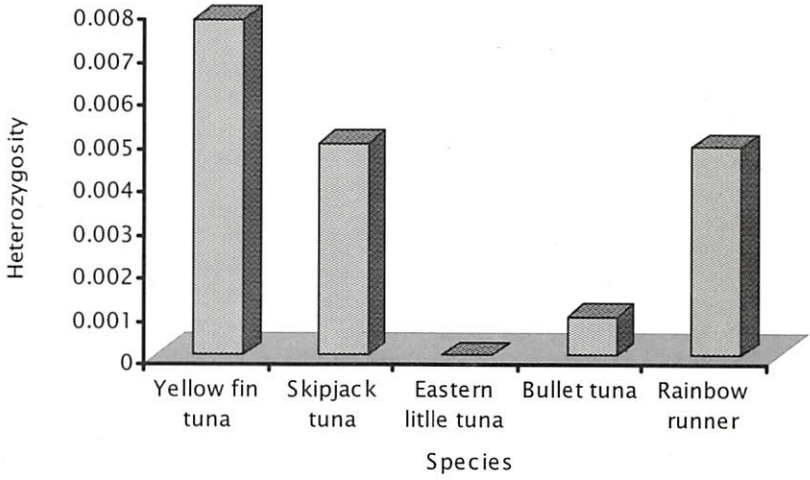

Figure 3. Experimental value of heterozygosity of five fish species

and diseases resistance), According to Leary *et al.* (1985) and Danzmann *et al.* (1985) heterozygosity value have positive correlation to growth of organism.

Difference existence of heterozygosity compiled gene character difference existence from each species which can cause by random genetic drift, selection and mutation.

GENETIC RELATIONSHIPS

Genetic relationship from bullet tuna (*A. rochei*) and eastern little tuna (*E. affinis*) having smallest value (0.001). Largest value obtained at rainbow runner fish (0.048). From this value designate among bullet tuna (*A. rochei*) and eastern little tuna (*E. affinis*) having closed genetic relationship, while yellow fin tuna, skipjack tuna, and runner rainbow was separated phylogenically. The mean F_{st} value at the third loci polymorphic of 0.004, indicated that about 4% of the total genetic diversity observed was due to population differentiation and that almost 86% was due to variation among individuals within populations. Illustration the inferred genetic relationship of the 5 species is pre-

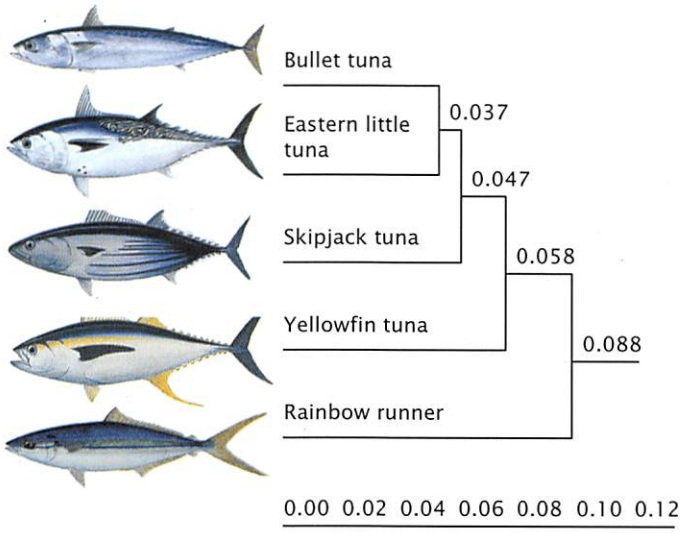

Figure 4. Dendogram showing relationships among 5 species based on values of genetic distance

sented as dendogram in Figure 4. According to the Unweighted Paired Group Method Arithmetic (UPGMA).

CONCLUSSIONS

1. Genetic markers of tuna and rainbow runner used as enzymes Idh* (*isocitrate dehydrogenase*), 6Pgd* (6 *phosphogluconate dehydroge nase*), Gpi* (*glucose phosphate isomerase*), Mdh* (*malate dehydrogenase*), Est* (*esterase*), and Sp* (*sarcoplasmic protein*) and rainbow runner (*E. bipunnulata*).
2. Yellowfin tuna had the highest heterozygosity value (0.008). Bullet tuna *(A. rochei)* and eastern little tuna *(E. affinis)* had close relation (0.00 1), while yellowfin tuna, skipjack and rainbow runner were separated by phylogeny.

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