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Molecular Electrophoretic Technique for Authentication of the Fish Genetic Diversity

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1. Introduction

Cobia (*Rachycentron canadum*) is the sole representative of their family, the Rachycentridae They are distributed worldwide in tropical and subtropical seas, as the Atlantic and Pacific Oceans (Miao, et al., 2009). There are several species, including cobia, seabream, red progy, snappers, scads and groupers that are raised by cage culture in Taiwan. Among these cage-cultured fishes, cobia certainly takes a leading distribution in both annual total production (81.9%) and total value production (75.4%) as compared to the rest in Taiwan (Fisheries Agency 2006).

Giant grouper (*Epinephelus lanceolatus*) are also found in tropical and subtropical waters from the Indo-Western Pacific Ocean. It is one of the two largest species of groupers in the world. Due to its fast growth and high price, giant grouper currently is regarded as a favorite species for marine culture in Taiwan (Hseu, et al., 2004).

Red coral trout (*Plectropomus leopardus*) a reef-associated fish in Western Pacific, distributed from southern Japan to Australia and eastward to the Caroline Islands (Zhang, et al., 2010). Only few studies concerning population genetics of *Plectropomus leopardus* has been reported.

All of cobia, giant grouper, and red coral trout are high-valued fish market in Taiwan and neighboring countries, including China, Japan, and Vietnam. For the globalization of the seafood industry, seafood authentication and food safety are very important. We must know that the source of fish or accurately species of the fish. Traditional method to distinguish the fish species was observed the external traits. It can cause the error judgment. Today, DNA-based methods are also more frequently employed for food authentication (Lockley and Bardsley, 2000). It has proven to be reliable, sensitive and fast for many aspects of fish species and food authentication. Asensio et al. (2009) were suggesting that the species-specific PCR method could be potentially used by regulatory agencies as routine control assay for the commercial grouper fillets authentication. PCR-based methods commonly used for fish species identification include PCR-sequencing, random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR). Those methods are simplicity, specificity and sensitivity.

Recently, many researchers have reported for the assessment of genetic structure of aquaculture species such as red mullet (Mullus barbatus), Tropical abalone (Haliotis asinina), and suminoe oyster (Crassostrea ariakensis) using several kinds of molecular markers (Garoia et al., 2004; Tang et al., 2004; Zhang et al., 2005; Maltagliati, et al., 2006), including RAPD, ISSR, AFLP, and RFLP methods. Many molecular methods are available for studying various aspects of wild populations, captive broodstocks and interactions between wild cultured stocks of fish and other aquatic species (Okumus and Ciftci, 2003). Among those methods, RAPD and ISSR technology were cheaper, simple, and fast. And just only one primer could obtain the different profiles for genomic analysis (Welsh and McClelland, 1990). RAPD is simple, rapid and cheap, it have high polymorphism. RAPD analysis has been used to evaluate genetic diversity for species, subspecies and population identification in common carp (Bártfai, et al., 2003), Indian major carps (Barman, et al., 2003). The microsatellite method was already used to study of genetic diversity of other grouper (Antoro, et al., 2006; Ramirez, et al., 2006; Wang, et al., 2007). Zeng, et al. (2008) have report that genetic analysis of Malaysia and Taiwan wild populations of giant grouper by microsatellite method. Their results were shown polymorphic loci in those populations, but they didn't discriminate the wild and cultivated populations of giant grouper. Beside, genetic markers can be suitable for assessing the differences between culture stocks and wild population and monitoring the changes in the genetic variation (Okumuş, et al., 2003). Monitoring the genetic diversity of natural populations and fish raised in fish hatcheries is fundamentally important for species conservation. Molecular markers can be very useful in this context (Povh et al., 2008).

In our study, we try to identify the seafood products, including cobia, giant grouper, and red coral trout from cultivated and wild populations by molecular markers, and provide the fish population genetic diversity for seafood management and good monitoring for brood stock management.

2. Methods and material

2.1 Fish sampling and genomic DNA extraction

14 giant grouper (*Epinephelus lanceolatus*) and 14 cobia (*Rachycentron canadum*) were collected from Southern Taiwan, as Penghu Island, Kaohsiung, and Pengtung during 2007-2009. Those were selected in different cultured farms and local markets. 14 of red coral trout (*Plectropomus leopardus*) were collected from Penghu Island during 2009-2011. All samples were described in Table 1. All specimens were confirmed in the laboratory.

Approximately 1g of fish muscle tissue samples were cut into small pieces and pulverized in liquid nitrogen. The powdered fish samples were obtained and extracted genomic DNA using the QIAGEN® DNeasy Blood kit (QIAGEN Inc., Valencia, California) according to manufacturer's instructions. The extracted DNA concentration in 200 µl of sterile water and then the quality of DNA were assessed by a Qubit™ Fluorometer (invitrogen, USA). The DNAs were stored at -20°C until PCR amplifications.

2.2 PCR-RAPD method

A total of 95 RAPD primers were used for PCR, which were shown in Table 2. Those sequences were obtained from University of British Columbia Biotechnology Laboratory,

No.	Species	Sampling sources	No.	Species	Sampling sources	No.	Species	Sampling sources
1	*E. lanceolatus*	Wild	**R1**	*R. canadum*	Wild	**71411**	*P. leopardus*	Wild
E2	*E. lanceolatus*	Cultivated	**R2**	*R. canadum*	Wild	**71412**	*P. leopardus*	Wild
E3	*E. lanceolatus*	Cultivated	**R3**	*R. canadum*	Cultivated	**71413**	*P. leopardus*	Wild
E4	*E. lanceolatus*	Cultivated	**R4**	*R. canadum*	Wild	**82011**	*P. leopardus*	Wild
E5	*E. lanceolatus*	Wild	**R5**	*R. canadum*	Cultivated	**91321**	*P. leopardus*	Wild
E6	*E. lanceolatus*	Cultivated	**R6**	*R. canadum*	Wild	**91711**	*P. leopardus*	Wild
E7	*E. lanceolatus*	Wild	**R7**	*R. canadum*	Cultivated	**91712**	*P. leopardus*	Wild
E8	*E. lanceolatus*	Wild	**R8**	*R. canadum*	Cultivated	**71421**	*P. leopardus*	Cultivated
E9	*E. lanceolatus*	Wild	**R9**	*R. canadum*	Wild	**71422**	*P. leopardus*	Cultivated
E10	*E. lanceolatus*	Cultivated	**R10**	*R. canadum*	Wild	**71423**	*P. leopardus*	Cultivated
E11	*E. lanceolatus*	Cultivated	**R11**	*R. canadum*	Wild	**81621**	*P. leopardus*	Cultivated
E12	*E. lanceolatus*	Cultivated	**R12**	*R. canadum*	Wild	**80622**	*P. leopardus*	Cultivated
E13	*E. lanceolatus*	Wild	**R13**	*R. canadum*	Wild	**81623**	*P. leopardus*	Cultivated
E14	*E. lanceolatus*	Wild	**R14**	*R. canadum*	Wild	**91322**	*P. leopardus*	Cultivated

Table 1. Specimens of *E. lanceolatus, R. canadum, and P. leopardus* fish analyzed and locality where they were collected

RAPD Analysis Kit (Amersham Pharmacia Biotech, Piscataway, NJ), and Operon primer kit (Operon, Advanced Biotechnologies). DNA amplification was performed in a final volume of 25 µl in the "Gene Amp PCR System 2720" thermal cycler (Applied Biosystems Inc., USA). The reaction mix contained 20 mM Tris-HCl, pH8.0, 50 mM KCl, 2 mM $MgCl_2$, 10 mM dNTPs each (dATP, dCTP, dGTP, dTTP), 20 µM of primer, 2.5 U *Taq*-polymerase (Promega, Co., Wisconsin, USA) and 1 µl of the 10 ng extracted DNA. The pre-amplification PCR procedure was: treatment at 94^{o}C for 5 min, followed by 35 cycles of denaturation at 94^{o}C for 30s, annealing at a primer-specific annealing temperature as table 2 for 30s and extension at 72^{o}C for 30s, and final extension at 72^{o}C for 10 min. The annealing temperature of Cobia was 36^{o}C. A 10 µl of the PCR product were analyzed in a 2 % agarose gel in 0.5 X TBE. The electrophoresis was performed at a constant voltage of 150 V for 150 min and 250 V for 1 min. The gel was stained with ethidium bromide and visualized under UV light.

2.3 PCR-ISSR method

ISSR primers of this study were listed in the Table 3. A total 59 primers were screened. Pre-amplification PCR reaction was conducted in 25 µl reaction containing 12.5 µl PCR master mix (Promega, Co., Wisconsin, USA), 1µl each primer, 1 µl of the 10 ng extracted DNA, and 10.5 µl dH_2O. Then, the mixtures were subjected to 94^{o}C for 5 min, followed by 35 cycles of denaturation at 94^{o}C for 30s, 30s at a primer-specific annealing temperature as table 3,

Primer	Sequence of primer (5′-3′)	Tm(℃)	references
RAPD16	GGTGGCGGGA	56	RAPD Primer Set#1, University of British Columbia
RAPD17	CCTGGGCCTC	56	
RAPD22	CCCTTGGGGG	56	
RAPD23	CCCGCCTTCC	56	
RAPD31	CCGGCCTTCC	56	
RAPD34	CCGGCCCCAA	56	
RAPD50	TTCCCCGCGC	56	
RAPD56	TGCCCCGAGC	56	
RAPD63	TTCCCCGCCC	56	
RAPD64	GAGGGCGGGA	56	
RAPD65	AGGGGCGGGA	56	
RAPD67	GAGGGCGAGC	56	
RAPD70	GGGCACGCGA	56	
RAPD71	GAGGGCGAGG	56	
RAPD73	GGGCACGCGA	56	
RAPD81	GAGCACGGGG	56	
RAPD83	GGGCTCGTGG	56	
RAPD84	GGGCGCGAGT	56	
RAPD86	GGGGGGAAGG	56	
RAPD87	GGGGGGAAGC	56	
RAPD88	CGGGGGATGG	56	
RAPD89	GGGGGCTTGG	56	
RAPD94	GGGGGGAACC	56	
RAPD95	GGGGGGTTGG	56	
RAPD96	GGCGGCATGG	56	
RAPD105	CTCGGGTGGG	56	RAPD Primer Set#2
RAPD106	CGTCTGCCCG	56	
RAPD115	TTCCGCGGGC	56	
RAPD149	AGCAGCGTGG	56	
RAPD157	CGTGGGCAGG	56	
RAPD158	TAGCCGTGGC	56	
RAPD173	CAGGCGGCGT	56	
RAPD174	AACGGGCAGC	56	
RAPD190	AGAATCCGCC	56	
RAPD196	CTCCTCCCCC	56	
RAPD198	GCAGGACTGC	56	
RAPD210	GCACCGAGAG	56	Set#3
RAPD211	GAAGCGCGAT	56	
RAPD218	CTCAGCCCAG	56	
RAPD241	GCCCGACGCG	56	
RAPD245	CGCGTGCCAG	56	
RAPD270	TGCGCGCGGG	56	
RAPD286	CGGAGCCGGC	56	
RAPD287	CGAACGGCGG	56	
RAPD319	GTGGCCGCGC	56	Set#4
RAPD480	GGAGGGGGGA	56	Set#5
RAPD534	CACCCCCTGC	56	Set#6
RAPD536	GCCCCTCGTC	56	

Primer	Sequence of primer (5′-3′)	Tm (℃)	references
RAPD540	CGGACCGCGT	56	Set#6
RPAD542	CCCATGGCCC	56	
RAPD563	CGCCGCTCCT	56	
RAPD571	GCGCGGCACT	56	
RAPD584	GCGGGCAGGA	56	
RAPD585	CCCGCGAGTC	56	
RAPD592	GGGCGAGTGC	56	
RAPD595	GTCACCGCGC	56	
RAPD598	ACGGGCGCTC	56	
RAPD601	CCGCCCACTG	56	Set#7
RAPD603	ACCCACCGCG	56	
RAPD606	CGGTCGGCCA	56	
RAPD615	CGTCGAGCGG	56	
RAPD620	TTGCGCCCGG	56	
RAPD625	CCGCTGCAGC	56	
RAPD626	CCAAGCCCGG	56	
RAPD640	CGTGGGGCCT	56	
RAPD647	CCTGTGGGGG	56	
RAPD769	GGGTGGTGGG	56	Set#8
RAPD770	GGGAGGAGGG	56	
RAPD771	CCCTCCTCCC	56	
RAPD772	CCCACCACCC	56	
primer1	GGTGCGGGAA	36	Ready-To-Go. RAPD Analysis Kit (Amersham Pharmacia Biotech, Piscataway, N J)
primer 2	GTTTCGCTCC	36	
primer 3	GTAGACCCGT	36	
primer 4	AAGAGCCCGT	36	
primer 5	AACGCGCAAC	36	
primer 6	CCCGTCAGCA	36	
OPA1	CAGGCCCTTC	56	(Operon, Advanced Biotechnologies)
OPA2	TGCCGAGCTG	56	
OPA3	AGTCAGCCAC	56	
OPA4	AATCGGGCTG	56	
OPA5	AGGGGTCTTG	56	
OPA6	GGTCCCTGAC	56	
OPA7	GAAACGGGTG	56	
OPA8	GTGACGTAGG	56	
OPA9	GGGTAACGCC	56	
OPA10	GTGATCGCAG	56	
OPA11	CAATCGCCGT	56	
OPA12	TCGGCGATAG	56	
OPA13	CAGCACCCAC	56	
OPA14	TCTGTGCTGG	56	
S514	CAGGATTCCC	56	Portman International (China) Limited, Hong Kong
S1036	AAGGCACGAC	56	
S1040	CCTGTTCCCT	56	
S1042	TCGCACAGTC	56	
S1201	CCATTCCGAG	56	

Table 2. RAPD primers of PCR amplification

extension at 72°C for 30s, and final extension at 72°C for 5 min before analysis by the electrophoresis as described previously.

Primer	Sequence of primer(5′-3′)	Tm(℃)	References
ISSR1	$(GGAC)_3A$	48	Pazza et al. (2007)
ISSR2	$(GGAC)_3C$	48	
ISSR3	$(GGAC)_3T$	48	
ISSR4	$(TGTC)_4$	48	
ISSR5	$(GGAC)_4$	48	
ISSR6	$(GGAT)_4$	48	
ISSR7	$(TAGG)_4$	48	
ISSR8	$(GACA)_4$	48	
ISSR801	$(AT)_8T$	50	Liu et al. (2006)
ISSR817	$(CA)_8A$	48	
ISSR825	$(AC)_8T$	48	
ISSR842	$(CA)_8YG$	52	
ISSR848	$(CA)_8RG$	51	
ISSR850	$(GT)_8YC$	51	
ISSR855	$(AC)_8YT$	50	
ISSR856	$(AC)_8YA$	49	
ISSR858	$(TG)_8RT$	48	
ISSR859	$(TG)_8RC$	52	
ISSR860	$(TG)_8RA$	51	
ISSR888	$BDB(CA)_7$	52	
SAS1	$(GTG)_4GC$	55	Maltagliati et al.(2006)
SAS3	$(GAG)_4G$	55	
UBC809	$(AG)_8G$	55	
UBC811	$(GA)_8C$	55	
UBC827	$(AC)_8G$	55	
IT1	$(CA)_8GT$	55	
IT2	$(CA)_8AC$	55	
IT3	$(GAG)_4AG$	55	
PT1	$(GT)_8C$	55	
ISSR807	$(AG)_8T$	48	UBC Primer Set#9, University of British Columbia
ISSR819	$(GT)_8A$	48	
ISSR822	$(TC)_8A$	48	
ISSR831	$(AT)_8YA$	48	
ISSR834	$(AG)_8YT$	48	
ISSR843	$(CT)_8RA$	48	
ISSR852	$(TC)_8RA$	48	
ISSR861	$(ACC)_6$	48	
ISSR862	$(AGC)_6$	48	
ISSR868	$(GAA)_6$	48	
ISSR871	$(TA)_8RG$	48	
ISSR873	$(GACA)_4$	48	
ISSR877	$(TGCA)_4$	48	
ISSR9	$(GAG)_5RY$	55	Hou *et al.*, 2006
ISSR10	$VBV(CA)_8$	54	
ISSR11	$VDV(GT)_8$	51	
ISSR12	$HVHT(GT)_7$	51	
ISSR13	$(CT)_8A$	49	
ISSR14	$(TG)_8GT$	48	
ISSR15	$(AG)_8TG$	54	
ISSR16	$(TC)_8C$	52	
ISSR17	$(TG)_8G$	53	
ISSR18	$(TG)_6R$	45	

ISSR19	$(CA)_6RY$	44
ISSR20	$(GT)_6YR$	44
ISSR21	$(GT)_6AY$	43
ISSR22	$(ACTG)_4$	52
ISSR23	$(GACA)_4$	48
ISSR24	$(CAC)_6$	57

Table 3. ISSR primers of PCR amplification

2.4 Genetic distances and phylogenetic analysis

Patterns from RAPD and ISSR methods were scored for the presence (1) or absence (0) of clear bands to analyze genetic similarities using the Dice coefficient of similarity. Similarity matrix cluster and phylogenetic analysis was used to reveal association among strains based on the unweighted pair group method with arithmetic averages (UPGMA) using the NTSYSpc software (Numerical taxonomy and multivariate analysis system, version 2.01b, State University of New York, Stony Brook, NY, USA) according to Rohlf (1997).

3. Results

3.1 RAPD method of giant group

A total of 14 giant grouper including cultivate and wild were obtained. Analysis on species of giant grouper in cultivate and wild. For RAPD method amplification, the species, For RAPD method, total 95 of RAPD oligonucletide primers were used to screening the genetic diversity of giant grouper. There are 21 RAPD primers (22.1%) have polymorphic bands. Total 279 bands were generated by those primers and 86 polymorphic bands (31%). The primer RAPD 115 (5′-TTCCGCGGGC-3′) was got the more diversity than other primers, have 8 polymorphic bands (Fig 1). The RAPD 245 primer was generated the less bands, only

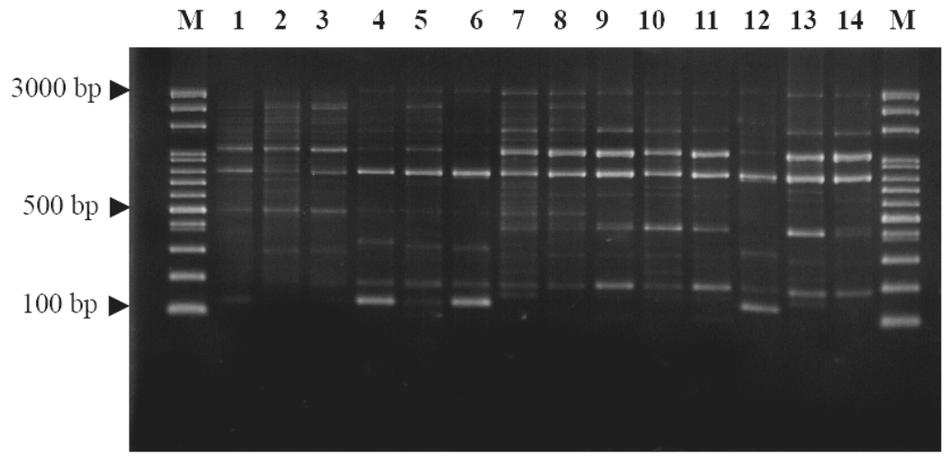

Fig. 1. RAPD profiles of the 14 *E. lanceolatus* fish obtained using the primer 115. Lanes M: Bio-100 bp DNA Ladder. Lanes 1-7: *E. lanceolatus* fish wild population (E1, E5, E7, E8, E9, E13, and E14); Lanes 8-14: *E. lanceolatus* fish cultivate population (E2, E3, E4, E6, E10 E11, and E12).

2 polymorphic bands. The sequence and PCR-RAPD condition were listed in Table 2. All primers were generated bands ranging in size from 100 to 3000 bp. The results shown that the ratio of polymorphic bands were between 13.3~66.7% by 21 RAPD primers. For dendrogram analysis, two groups were identified by RAPD 115 primer (Fig 2). E1, E5, E14, E7, E8, and E9 samples were clustered in group I, which were collected from wild population. Group II, which including E13, E10, E2, E3, E4, E6, E11, and E12 samples. For Group II, all samples were belonged to cultivated populations. For giant grouper, wild (seven samples) and cultivated (seven samples) populations of giant grouper can be discriminated by RAPD method.

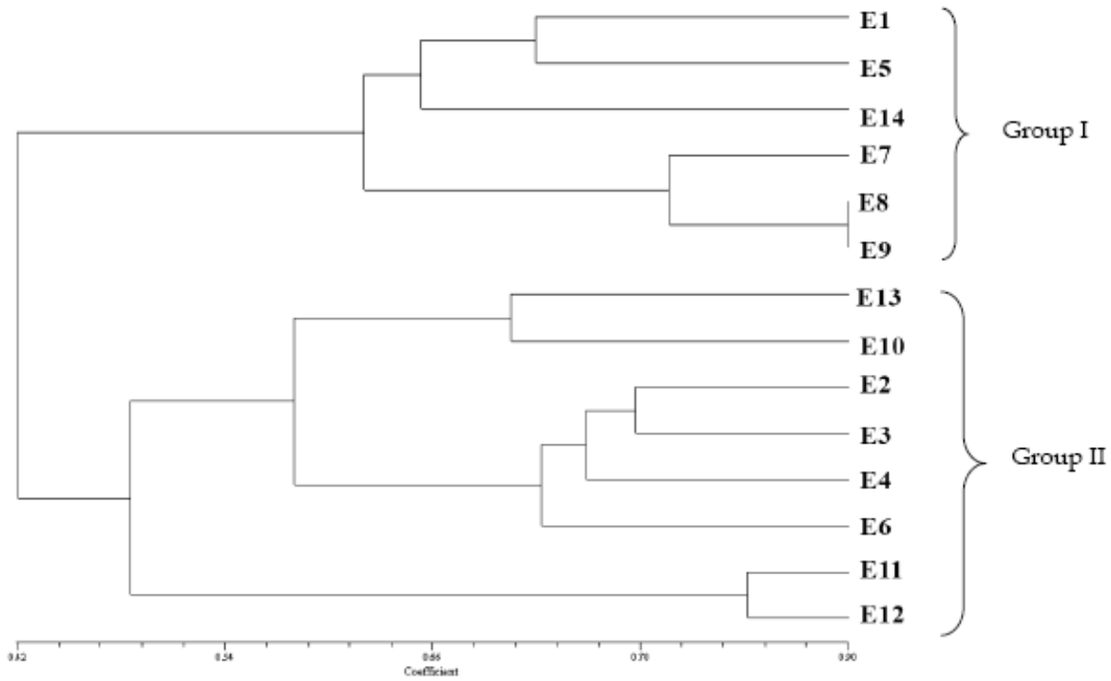

Fig. 2. UPGMA consensus dendrogram of dissimilarity among individuals analyzed using the primer RAPD 115.

3.2 ISSR method of giant group

Results of ISSR analysis, 59 primers were used in this study. According the results of ISSR method, 17 primers (29%) have polymorphic patterns. Total 166 bands were generated, 58 polymorphic bands (34.9%). The primer ISSR IT3 was got the more diversity than other primers, have 20 bands. The ISSR 15 primer was generated the less bands, only 3 bands. All the polymorphic patterns were ranged between 100~3000 bp. ISSR primer 868 (5′-$(GAA)_6$-3′) was better distinguished than other primers. The result was shown in Fig 3. For giant grouper, the patterns of ISSR primer868 could discriminate giant grouper between wild and cultivated populations. For dendrogram analysis, four groups were clustered by ISSR primer868 primer (Fig 4). Among those groups, Group I, Group III, and Group IV were collected from wild population. Samples were clustered in Group II were from cultivated populations. We also found that the results of ISSR method have the same tend to RAPD method. ISSR method was more discriminate ability than RAPD method.

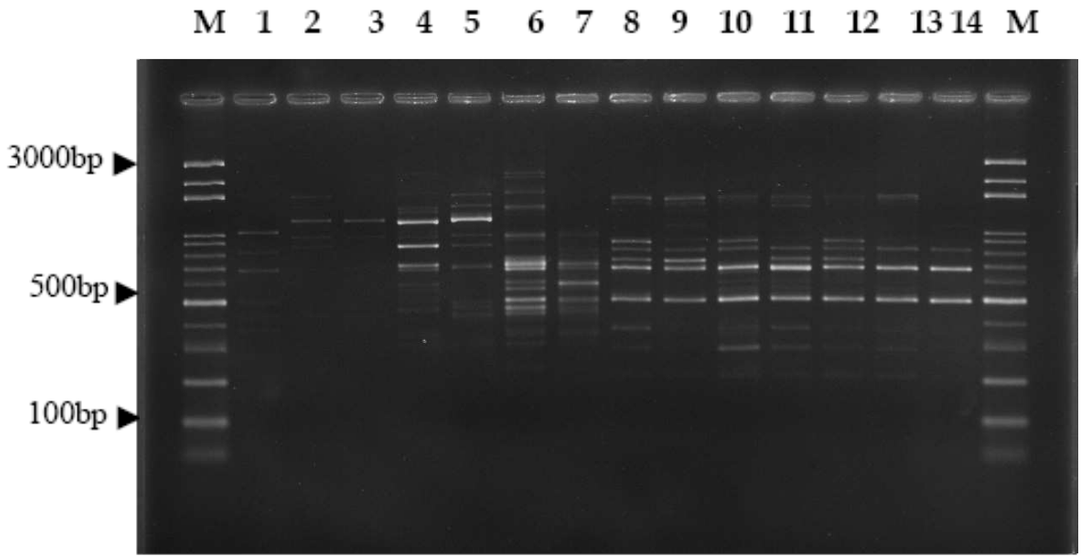

Fig. 3. ISSR profiles of the 14 giant grouper fish obtained using the primer ISSR 868. Lanes M: Bio-100bp DNA Ladder. Lanes 1-7: giant grouper fish wild population (E1, E5, E7, E8, E9, E13, and E14); Lanes 8-14: giant grouper fish cultivated population (E2, E3, E4, E6, E10, E11, and E12).

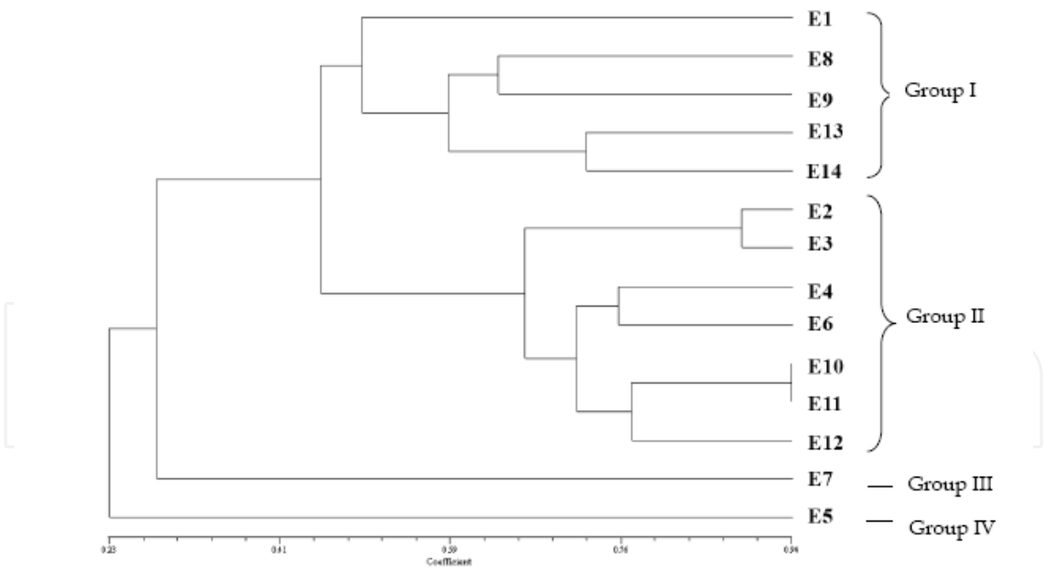

Fig. 4. UPGMA consensus dendrogram of dissimilarity among individuals analysed using the primer ISSR 868.

3.3 RAPD and ISSR methods of cobia

Ninety-five RAPD primers and 59 ISSR primers were used for PCR amplification. The results were shown that all the cobia samples were the same patterns and no polymorphic

bands. The primer ISSR UBC809 and RAPD31 were generated 12 to 17 bands and in size from 100 to 2000 bp. The results were also shown in Fig 5 and Fig 6.

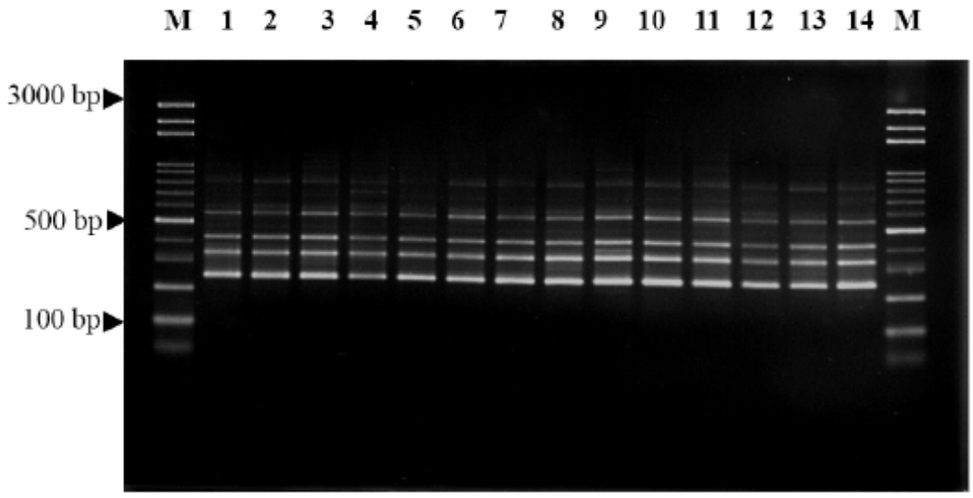

Fig. 5. ISSR profiles of the 14 *R. canadum* fish obtained using the primer UBC 809. Lanes M: Bio-100 bp DNA Ladder. Lanes: 1, 2, 4, 6, 9, 10, 11, 12, 13, and 14 (R1, R2, R4, R6, R9, R10, R11, R12, R13, and R14) *R. canadum* fish wild population; Lanes: 3, 5, 7, and 8 (R3, R5, R7, and R8) *R. canadum* fish cultivate population.

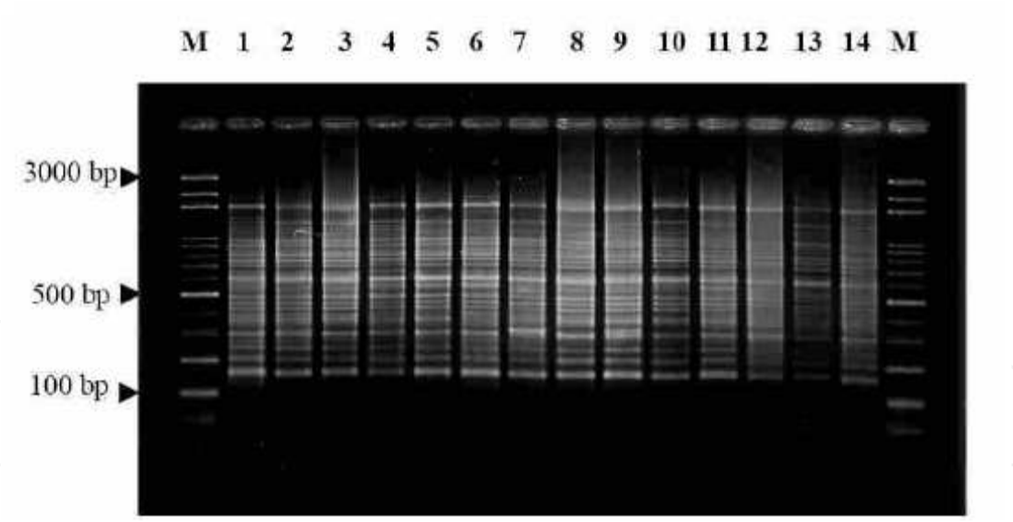

Fig. 6. RAPD profiles of the 14 *R. canadum* fish obtained using the primer 31. Lanes M: Bio-100 bp DNA Ladder. Lanes : 1, 2, 4, 6, 9, 10, 11, 12, 13, and 14 (R1, R2, R4, R6, R9, R10, R11, R12, R13, and R14) *R. canadum* fish wild population; Lanes: 3, 5, 7, and 8 (R3, R5, R7, and R8) *R. canadum* fish cultivate population.

Sequence variability of mitochondrial DNA regions was low between the six cobia (Garnet, et al., 2002). These results were also similar in our study. Both RAPD and ISSR methods have no different patterns. Hence, this could provide more useful information of molecular genetic data in population and stock enhancement studies.

3.4 ISSR methods of red coral trout

For screening ISSR primers, the ISSR primer15 (ISSR15: 5′-$(AG)_8$TG -3′) was better distinguished than other primers. The result was shown in Fig 7. The primer ISSR 15 was generated 10 to 16 bands and in size from 200 to 2000 bp. For dendrogram analysis, three groups were identified. Group I, the 71412, 71413, 71422, and 82011 were clustered in the group. Group II, including 81621, 91321, 71411, and 91712 samples; group III including 71423, 80622, 91322, 71421, and 81623 samples. All the nodes of the dendrograms ranged from 90 to 100%. The result was shown in Fig 8.

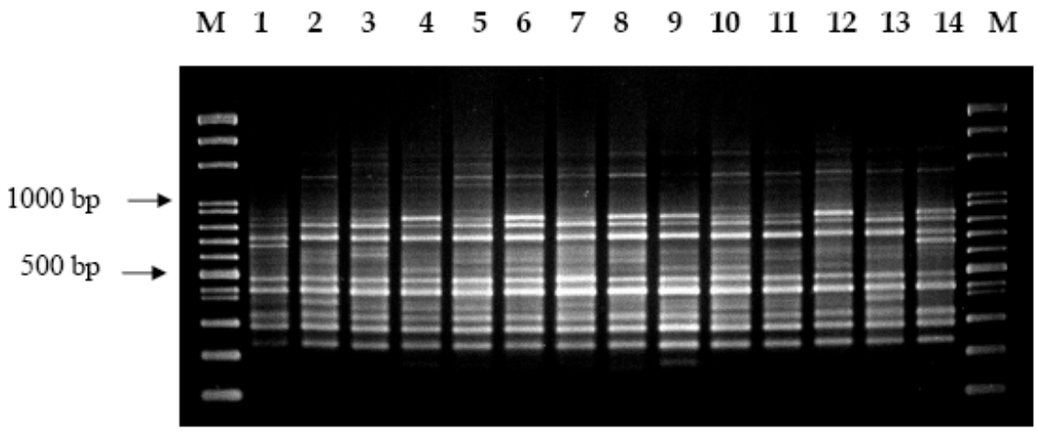

Fig. 7. ISSR profiles of the 14 red coral trout obtained using the primer ISSR 15. Lanes M: Bio-100 bp DNA Ladder. Lanes: 4, 5, 6, 8, 9, 10, and 12 (71411, 71412, 71413, 82011, 91321, 91711, and 91712) red coral trout fish wild population; Lanes: 1, 2, 3, 7, 11, 13, and 14 (71421, 71422, 71423, 81621, 80622, 81623, and 91322) red coral trout fish cultivate population.

4. Discussions

RAPD and ISSR methods were generally used for genetic diversity and populations study; those methods also could be used to analyze the breeding relationship. For species identification and genetic resource/diversity analysis, RAPD and microsatellitsa method were recommended (Liu & Cordes, 2004). The RAPD techniques has been used for discrimination of populations of species of the genus Barbus, grouper, Nile perch and wreck fish, salmonids, among others (Partis & Wells, 1996; Callejas & Ochando, 2001; Asensio et al., 2002; Jin, Cho, Seong, Park, Kong & Hong, 2006). Genetic analysis with RAPD markers is relatively easy, fast, and efficient. RAPD analysis, however, may not be practical for identifying interbreed species (Martinez, Elvevoll & Haug, 1997). SSRs are inherited in a co-dominant fashion. This allows one to discriminate between homo- and heterozygous state, and increases the efficiency of genetic mapping and population genetic studies. ISSR markers have recently been used successfully for genetic analysis in hatchery and wild *Paralichthys olivaceus* strains. It indicates that molecular marker systems contribute greater levels of capability for the detection of polymorphism, and provide a better solution for the assessment of genetic variations (Shikano, 2005; Liu, Chen, Li & Li, 2006).

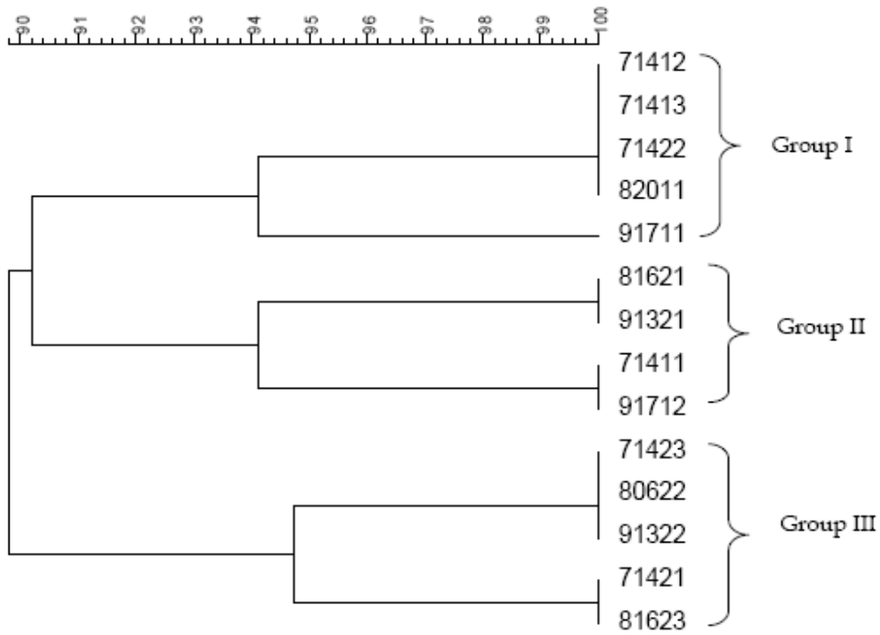

Fig. 8. UPGMA consensus dendrogram of dissimilarity among individuals analysed using the primer ISSR 15.

According to our results, RAPD and ISSR method can be effectively discriminate giant grouper from different sources, such as cultivate and wild, even if fish are from different cultivate farms. But cobia and red coral trout were less discriminate ability. The study found that ISSR and RAPD methods were positively high correlations. Giant grouper species have highly genetic diversity. A comparison of RAPD and ISSR patterns in 14 giant grouper samples, ISSR primers have higher polymorphism and fewer bands than those of RAPD primers. It could provide simple and convenient method to discriminate genetic variation of giant grouper samples. In this study, ISSR method could distinguish genetic variation within specie and different populations. Some reports also have suggested that ISSR may reveal a much higher numbers of polymorphic fragments per primer than those of RAPD (Esselman, et al., 1999). Among these markers, microsatellite DNAs have revolutionized the use of molecular genetic markers in the applications mentioned before, and the markers are destined to dominate this type of studies in the coming years (Asensio, 2007). It also has been revealed as important tools in studies regarding the genetic structure of populations, phylogeographic relations and phylogenetic reconstruction in fish (Antunes, et al., 2010).

5. Conclusion

We developed DNA molecular marker techniques which could be used to generate information for fish genetic diversity, species identification, trace genetic variation between different individuals in aquaculture, authenticate fish, fishery products and provide good reference resources for species sources and relationships.

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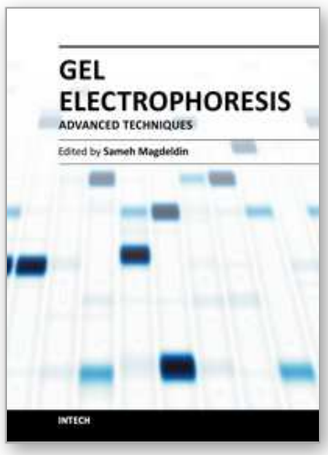

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