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Genetic variations and phylogenetic relationship among snakehead fish, *Channa Striatus* (Actinopterygii: Perciformes: Channidae) population based on RAPD-PCR analysis

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

Evolutionary study is an important parameter to assess the stocks via selective breeding programs. Hence, establishing molecular markers for genetic programs to address the issues related population management studies, especially identification and discrimination is required. In this study, using random amplified polymorphic DNA (RAPD) -polymerase chain reaction (PCR) technique, genetic variations and phylogenetic relationship among population of snakehead fish, *Channa striatus* from four states (Perak, Penang, Kedah and Perlis) of Peninsular Malaysia were examined. Live and healthy fishes were collected at each of the sampling locations. Genomic DNA was isolated from the caudal fin of the fish and quantified. To amplify the DNA, preliminary screening was carried out with 20 decamer OPA primers, where only 3 primers (OPA-2, OPA-3, and OPA-13) were produced clear and reproducible bands and these primers were selected for the genetic variation analysis. Results showed that the genetic distance between population ranged from 0.0588 to 0.2272; and individuals within the populations were closely related at an average genetic distance of 0.18. Furthermore, dendogram construction using un-weighted pair-group method with arithmetic average (UPGMA) revealed the close relationship between snakehead fish sampled from Kedah and Perak states, thus indicating less variation among the populations in the north-western states of Peninsular Malaysia. The slight variation in the genetic diversity within and between populations in the north-west region of Peninsular Malaysia could be attributed to over-fishing and natural destruction of fish habitat, which may lead to inbreeding and genetic drift. Based on the obtained results it is concluded that RAPD-PCR technique is a reliable method to successfully investigate the genetic variation among fish population.

KEYWORDS

Snakehead; *Channa striatus*; RAPD-PCR; RAPD marker; genetic variation; DNA

1. INTRODUCTION

Genetic variation is a key component for improving the stock through selective breeding programs (Rahman et al., 2009). Therefore the use of molecular genetic markers to address the questions related to the aquaculture management has found a steadily widening application in the last two decades. These markers can provide valuable information for various aspects of aquaculture practice, such as genetic identification and discrimination of aquaculture stocks, monitoring the consequences of founding and propagation of aquaculture stocks, assisting selective

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breeding programs and assessing chromosomal and gene manipulations such as induction of polyploidy and gynogenesis (Ferguson et al., 1993). Another important application of genetic markers in aquaculture is the assessment of the impact on natural populations which escaped or released into culture system. Information on the genetic structure of cultivable fish and shellfish species is useful to optimize the stock identification, stock enhancement, breeding programs, management for sustainable yield and preservation of genetic diversity (Dinesh et al., 1993; Garcia and Benzie 1995; Tassanakajon et al., 1997). Studies based on the population diversity in the genetic make-up of species were carried out using various techniques, including randomly amplified polymorphic DNA (RAPD) (Rahman et al., 2009).

RAPD analysis is a technique based on the polymerase chain reaction (PCR) amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsh and McClelland 1990, Williams et al., 1990; Barman et al., 2002; Liu and Cordes, 2004). This fingerprinting method was first described by Welsh and McClelland (1990) and Williams et al., (1990) as it is a process in which random segments of DNA in the genome were amplified through PCR by using single primers of arbitrary nucleotide sequence, usually about 10 nucleotides in length. The method provides information on rapid and convenient assessments of similarities and differences in the genetic compositions of related individuals (Kazan et al., 1993). It is very simple technique which required no prior knowledge on the genetic make-up of the organism (Hadrys et al., 1992). RAPD fingerprinting has been used successfully in recent times by many researchers for the analysis of phylogenetic relationship among various organisms (Stiles et al., 1993; Bardakci and Skibinski, 1994; Orozco-Castillo et al., 1994; Van Rossum et al., 1995). This technique has also been used to evaluate the genetic diversity of species and subspecies in guppy (Dinesh et al., 1993), tilapia (Bardakci and Skibinski, 1994; Dinesh et al., 1996), catfish (Liu et al., 1998; Almeida et al., 2003), common carp (Bartfai et al., 2003) and Indian major carps (Barman et al., 2002). However, in spite of the growing commercial importance of snakehead fish *Channa striatus*, no information is yet reported on its genetic structure, similarity or variability from different locations worldwide.

C. striatus is native to many Asian, Asia-Pacific and African countries. It is reported to live in rivers, lakes, swamps, paddy fields and mining pools. This species is air-breathing in nature; also it is a highly-prized freshwater food-fish (Marimuthu et al., 1999; Arockiaraj et al., 2004).This fish has fine white flesh with a few intra-muscular bones; and has an excellent taste when the fish is consumed. Eating this fish is also believed to enhance the post-surgery recuperation, as the flesh is believed to contain substances that help in rejuvenating several illnesses (Wee, 1982; Lee and Ng, 1991). It is therefore, served especially to elderly people and women during convalescence and after child delivery (Ling, 1977). Yaakob and Ali (1992) and Baie and Sheikh (2000) have also noted the importance of snakehead for hastening the healing of wounds and internal injuries due to the presence of certain fatty acids such as prostaglandin and thromboxin. Presently, snakeheads are quickly gaining commercial prominence due to its medicinal value in Asian markets, even though the medical potentials and applications may vary considerably within the species. Information exists on snakehead breeding (Haniffa et al., 1999; Haniffa et al., 2002; Marimuthu et al., 2001a; 2007) spawning behaviour (Marimuthu et al., 2001b), embryonic and larval development (Marimuthu and Haniffa, 2007). The objective of this study was therefore, to investigate the likelihood of genetic variability using RAPD analyses among population of *C. striatus* sampled at various locations from Peninsular Malaysia.

2. MATERIALS AND METHODS

2.1. Fish sampling and DNA extraction

A total of 12 fishes (weight ranged between 500 g and 1 kg) were randomly selected from the populations at designated sampling points in Peninsular Malaysia (Perlis, Perak, Penang and Kedah as shown in Figure 1) and transported to the research facility at the University. Thus, 3 fishes were used per location for RAPD analysis. Using sharp and sterilized laboratory blades and forceps, fresh caudal fin tissue were dissected out from the fish and immediately stored in 95% ethanol followed by storage in the refrigerator at 4 °C until DNA extracted.

Genomic DNA was extracted from the tissue using AquaGenomic kit (AquaGenomicTM, USA) as per the extraction protocol provided by the supplier. The concentration of DNA was determined using DU 800 UV-VIS spectrophotometer (Beckman Coulter, USA). The purity of the genomic DNA was estimated by calculating the ratio of the optical density at 260 and 280 nm, respectively. Then, the extracted DNA was diluted at concentration 25 ng/μL using deionized water, and subsequently used for PCR amplification.

Figure 1. Map showing the sampling locations of *C. striatus* populations in Peninsular Malaysia. Red star indicates the sampling locations.

2.2. DNA amplification

Twenty commercially available decamer primers (OPA 1 to OPA 20) were obtained from First BASE Laboratories, Malaysia (Table 1) and they were subsequently used in this study (Nagarajan et al., 2006). DNA amplification was performed in a total reaction volume of 25 µL which contains 25 ng of template (genomic DNA), 1X PCR buffer, 3.5 mM $MgCl_2$, 0.5 mM dNTPs and 4U Taq DNA polymerase. Likewise, control reactions were prepared without genomic DNA. The reactions were performed on a programmable MyCycler Personal Thermal Cycler (BIO-RAD, USA) as follows: 95 °C pre-denaturation for 1 min, followed by 35 cycles of denaturation for 30 sec each at 94 °C, 30 sec of annealing at 36 °C and 1 min of extension at 72 °C. The reaction was then subjected to a final extension at 72 °C for 2 min. The obtained amplified DNA was considered as PCR products for the subsequent evaluations. To amplify the DNA, preliminary screening was carried out with 20 decamer OPA primers, where only 3 primers (OPA-2, OPA-3, and OPA-13) were produced clear and reproducible bands and these primers were selected for the genetic variation analysis.

Table 1. Primer code, sequence, nucleotide length and G+C content of primers used in RAPD analysis

No.	Primer code	Primer sequence (5' – 3')	Nucleotide length	G+C content (%)
1.	OPA-01	CAGGCCCTTC	10-mer	70
2.	OPA-02	TGCCGAGCTG	10-mer	70
3.	OPA-03	AGTCAGCCAC	10-mer	60
4.	OPA-04	AATCGGGCTG	10-mer	60
5.	OPA-05	AGGGGTCTTG	10-mer	60
6.	OPA-06	GGTCCCTGAC	10-mer	70
7.	OPA-07	GAAACGGGTG	10-mer	60
8.	OPA-08	GTGACGTAGG	10-mer	60
9.	OPA-09	GGGTAACGCC	10-mer	70
10.	OPA-10	GTGATCGCAG	10-mer	60
11.	OPA-11	CAATCGCCGT	10-mer	60
12.	OPA-12	TCGGCGATAG	10-mer	60
13.	OPA-13	TCTGTGCTGG	10-mer	70
14.	OPA-14	TCTGTGCTGG	10-mer	60
15.	OPA-15	TTCCGAACCC	10-mer	60
16.	OPA-16	AGCCAGCGAA	10-mer	60
17.	OPA-17	GACCGCTTGT	10-mer	60
18.	OPA-18	AGGTGACCGT	10-mer	60
19.	OPA-19	CAAACGTCGG	10-mer	60
20.	OPA-20	GTTGCGATCC	10-mer	60

A, Adenine; T, Thymine; G, Guanine; C, Cytosine

2.3. Statistical analysis

The DNA profile or fingerprint of each fish population was documented. Patterns of the RAPD were visually analyzed and scored from the gel images. All the distinctly amplified fragments were assigned identification numbers and scored separately for each sample and primer based on its presence (1) or absence (0) in each population, where only reproducible and distinctly amplified fragments were scored (Nagarajan et al., 2006). A data matrix was subsequently generated, and each individual's fingerprint was constructed based on the following criteria: where a given amplified fragment present in an individual, it was assigned either 1 or 0 when the fragment was absent. Genetic similarity indices (SI) were calculated from Dice formula in RAPDistance Package Software (Ver. 1.04) (Dice, 1945; Nei and Li 1979; Amstrong et al., 2004). Genetic similarity indices values were used to construct the unweighted pair-group method with averages (UPGMA) dendograms, using PHYLIP (Ver. 3.69), to determine the genetic distance between the four populations taken for analysis.

3. RESULTS AND DISCUSSION

The present study utilized RAPD marker technique to investigate the occurrence of phylogenetic relationships and intra-specific genetic variations between four populations of *C. striatus* from Peninsular Malaysia. From the results of the present analysis, the purity of the extracted genomic DNA of the fish sample varied from 1.83 to 2.01, whereas the concentration ranged between 0.034 and 0.049 μg/μL. The quality and quantity of extracted genomic DNA are important factors that have effects on RAPD reproducibility. As random primer is capable to amplify very small quantity of DNA from any organism, DNA purity is important, to obtain clear bands. DNA concentration also plays a major role in the reproducibility of RAPD markers, where large volumes of DNA have been reported to inhibit the amplification due to competition of the primer for the DNA template (Fraga et al., 2005).

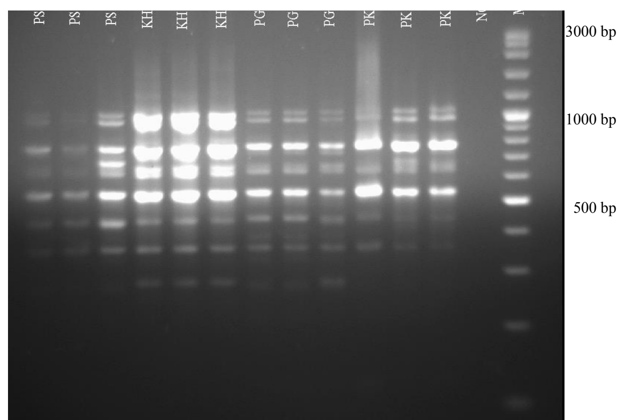

Figure 2. RAPD patterns of *C. striatus* generated by primer OPA-2 (Lanes: PS1-PS3, Perlis population; KH1-KH3, Kedah population; PG1-PG3, Penang population; PK1-PK3, Perak population; NC, negative control; M, Marker).

In the present investigation, 20 different decamer primers were screened for their suitability to be utilized in the RAPD analysis. Screening of primers is an important prerequisite process, thus can be used as DNA markers to identify the species or to investigate for genetic variations within and between species. Among the 20 primers tested, three (OPA-02, OPA-03 and OPA-13) were noted to generate reproducible bands, whereas the remaining 17 did not amplify or produced highly inconsistent amplification products from the same individuals and were therefore, excluded from further analysis. In other words, primers were selected based on the ability to produce well-defined and reproducible banding patterns. Figure 2, 3 and 4, respectively showed the amplification patterns generated with OPA-02, OPA-03 and OPA-13 primers. From the results as shown in the Figures 2, 3 and 4, a total of 28 reproducible bands were obtained for the three primers among the four populations evaluated. The number of detected amplified bands was varied depending on the primers, population and individuals. The number of amplified bands was 3-8 in OPA-02, 8-10 in OPA-03 and 7-10 in OPA-13. The sizes of the amplified bands ranged between 500 and 3530 base pairs. Generally, number and sizes of the bands generated were dependent on the nucleotide sequences of the primers used and sources of the template DNA, thus resulting genome-specific fingerprints of the random DNA bands was consistent with the earlier report of Welsh et al., (1991).

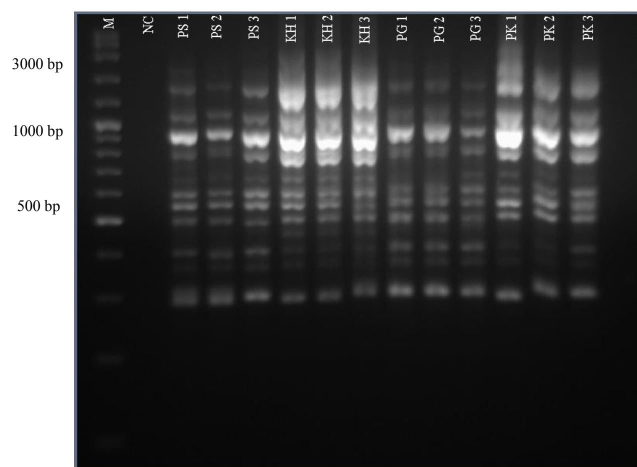

Figure 3. RAPD patterns of *C. striatus* generated by primer OPA-3 (Lanes: M, Marker; NC, negative control; PS1-PS3, Perlis population; KH1-KH3, Kedah population; PG1-PG3, Penang population; PK1-PK3, Perak population).

The presence of genetic variability within species (among populations and also between individuals in the same population) is essential for their survival and successful response to environmental changes (Nagarajan et al., 2006). Among the four populations analyzed (Table 2), highest genetic similarity was recorded between population from Kedah and Perak (0.0600), whereas the lowest was detected between Perak and Perlis (0.1739). Further on the basis of genetic identity, clustering analysis revealed that the genetic relationship among the populations which is described as dendogram (Fig. 5). It appears that the Kedah population is genetically closer to the Perak population than the populations from Penang and Perlis.

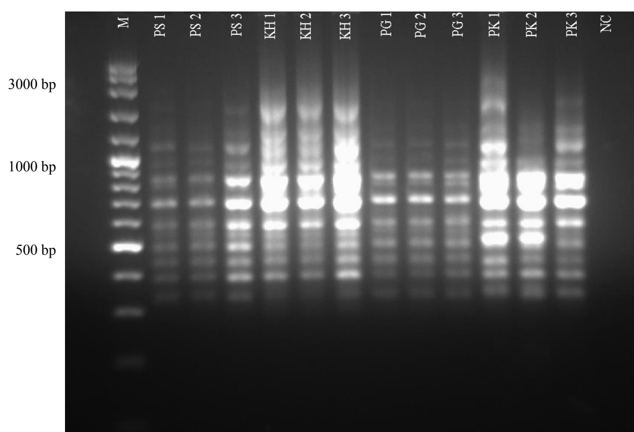

Figure 4. RAPD patterns of C. striatus generated by primer OPA-13 (Lanes: M, Marker; PS1-PS3, Perlis population; KH1-KH3, Kedah population; PG1-PG3, Penang population; PK1-PK3, Perak population; NC, negative control)

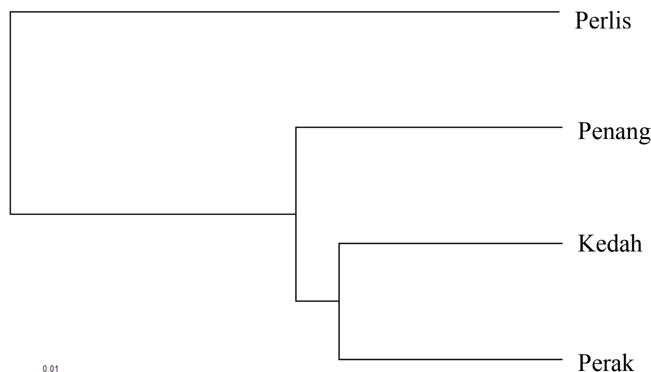

Figure 5. UPGMA dendrogram among four populations of C. striatus based on average linkage cluster analysis using RAPD markers.

Table 2. The matrix of similarity index among four populations of *C. striatus*

States	Perak	Penang	Kedah	Perlis
Perak	0.0000	-	-	-
Penang	0.0638	0.0000	-	-
Kedah	0.0600	0.0612	0.0000	-
Perlis	0.1739	0.1363	0.1162	0.0000

Genetic distance between populations determined using Nei and Li (1979) indices showed that the genetic distance below 0.1, thus suggested a significant correlation between genetic identity and geographical distance. This is consistent with the conclusions of Ikeda et al., (1993) who reported that the population structure of freshwater organisms is dependent on the distribution of river systems.

4. CONCLUSIONS

In conclusion, results of the present study showed that among the 4 populations studied, Perak and Kedah populations were genetically (by RAPD-PCR analysis) closer to each other (likely due to geographical locations), than the populations from Perlis and Penang. Further molecular studies may be required to confirm the presently established phylogenetic relationships existing among snakehead fish in different parts of Malaysia.

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