Development of DNA Markers for Identification of Colour Varieties and Sex in Tiger Barbs

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Introduction

The beautiful and exotic colours of ornamental fish have led an interest to breed and study many species of ornamental fish. The use of molecular techniques has becoming very popular in the study of stock identification which is very useful to aquaculture and fishery management. Ornamental fish industry plays an important role in the fisheries sector in Malaysia and many local species such as Harlequin fish (*Rasbora heteromorpha*), Arowana (*Scleropages formosus*) and Tiger barb (*Puntius tetrazona*) has potential for commercial value. Phylogenetic analysis and genetic variability of the fish had been widely used in fisheries research, as it is technically simple, fast and economical for determining the levels of polymorphism within stocks and between stocks.

The objectives of the projects are to determine the genetic markers of tiger barbs using RAPD and MtDNA analysis, as these will minimise the incorrect identification and stock contamination for commercial purposes.

Materials and Methods

Genetic variability between and within three varieties of domesticated Tiger barb was studied using random amplified polymorphic DNA (RAPD) markers and MtDNA analysis. Fifty random individuals of each variety (normal, green and yellow) and twelve of 9-10 mer random primers with 50-77.7% of G+C content were used to generate the RAPD markers separable by agarose gel electrophoresis. Isolation of genomic DNA and analysis of mtDNA were conducted using muscle tissue following the procedures by Pure-Gene Tissue Kit II (BST TechLab). Two portion of the mtDNA gene were amplified. Primer 16SL (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SH (5'-CCG GTC TGA ACT CAG ATC ACG T-3') were used to amplify a partial region of 16S rDNA (about 620 bp). The second portion was obtained from the 12s ribosomal RNA and tRNA-Val gene (about 730 bp) was amplified using the following primer pair: OMT16SF ('5 – TGC CAG CCA CCG CGG TTA TAC CT – 3') and tRNA02 (5' – GGA TGT CTT CTC GGT GTA AG – 3') (Saiki *et al.*, 1988). The temperature regimen for 30 cycles was 1 min at 95°C, 1 min at 55°C and 2 min at 70°C. The amplified products were purified and concentrated by enzyme kit (PCR product Pre- Sequencing Kit, Pharmacia Biotech); the PCR product was mix with the mixture of SAP, EXON I, deionised water and incubated at 37°C for 15 minutes followed by 80°C for 15 minutes. The sequence amplification was performed with the Big Dye Terminator cycler sequencing kit (ABI, Pharmacia Biotech); the purified products were mixed with 1 µl reaction mix and 0.5 µl primer (for each reaction the duplicate for forward and reverse primer was prepared). The reaction mixture was performed with the parameter of 25 cycle at 96°C for 30 second, 15 second at 50°C and 4 min at 60°C. Purification of sequence reaction of PCR product was done with the mixture of 3M sodium acetate and 95% ethanol (2:30). Then the mixture was mixed with the PCR product and incubated at room temperature before it was centrifuged at 15,000 rpm for 20 minutes and mixed the pellet with 70% ethanol. Formamide was added into the sample and it was then analyzed using automated sequencer of ABI prism 310 genetic analyzer (Perkin-Elmer).

The analysis of the multiple alignments of sequences was done using the Clustel V program with default settings (Higgins and Sharp, 1988). Computer generated alignments were compared to secondary-structure models described by Neefs *et al.*, (1991) and Orti *et al.*, (1996). The PHYLIP ver. 3.572 package and PAUP* 4.0 were used for the phylogenetic analyses. Genetic distance was calculated using the Jukes et al.-Cantor model (Jukes et al. and Cantor, 1969), Kimura's et al.two parameter (K2) model (Kimura et al., 1980) and a neighbor joining trees (Saitou et al.and Nei, 1987) was constructed.

The RAPD markers were analysed using Nei and Li's Similarity Index (SI) in which bands were scored as present or absent for each variety and variation among varieties was quantified using the index of dissimilarity. The same variety from different states was grouped in the same cluster.

Results and Discussion

A total of 73 RAPD markers were found in the normal variety, 79 in the green variety and 84 in the yellow variety with an average of 6-7 markers per primer. Each variety of fish was found polymorphic with the normal variety identified as the most polymorphic (50.57%), followed by the yellow (45.95%) and the green was the least polymorphic (40.68%). Total DNA from three varieties of tiger barb was amplified at 2 region of mtDNA gene using two set of primer (16SL/16SH and OMT16SF/ tRNA02). Primer 16SL and 16SH amplified at 620 bp while primer OMT16SF and tRNA02 amplified at 750 bp and the consensus sequence for a partial region of the 16S rDNA gene and 12s ribosomal RNA and tRNA-Val gene in Tiger barb was 500 bp and 539 bp respectively.

Conclusions

The RAPD analysis suggests that the low polymorphisms among individuals of the same variety indicate a high level of inbreeding among the fish populations. There is a close genetic relationship between the normal and the green variety compared to the

yellow variety. The MtDNA study is a specific study breeding program can be formulated to increase the genetic variability within the varieties and populations using varieties with high SI and lower SI (green and yellow varieties).

Benefits from the study

The availability of genetic markers to identify fish stocks as well as individual fishes will enable commercial breeders to have propriety tags of their cultural strains. Examination of mtDNA has indicated that certain areas of the genome may be more variable than others and maximum amount of information can be gained by sequencing the mtDNA fragment. Though at present, this is an expensive and time consuming procedure, but this gives more information than RAPD analysis. It will allow fish seed to be monitored the levels of genetic variation in the broodstocks to avoid deleterious effects of inbreeding.

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I·R·P·A INTENSIFICATION OF RESEARCH IN PRIORITY AREAS

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