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APPLICATION OF RAPD TECHNIQUE IN FISH: I- DETECTION OF POLYMORPHISM AMONG THREE GENERA (TILAPIA, SAROTHERODON AND OREOCHROMIS) AND BETWEEN TWO SPECIES (OREOCHROMIS AUREUS AND OREOCHROMIS NILOTICUS) OF TILAPIA.

BY

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ABSTRACT

Random amplified polymorphic DNA (RAPD) analysis was applied to the four-tilapia species (three genera): redbelly tilapia (Tilapia zillii), white tilapia (Sarotherodon galilaeus), blue tilapia (Oreochromis aureus) and Nile tilapia (Oreochromis niloticus). Fifteen random 10-mer and five 20-mer primers were used to assay polymorphisms among three genera and between two species. Different RAPD fragment patterns were observed for different genera and different species. Results showed that there are great differences among the three genera of tilapia fish. Also, it was found specific marker can be discriminate O. niloticus from the other species. Moreover, data demonstrated that RAPD marker was useful for systematic investigation at the level of tilapia species.

INTRODUCTION

Aquaculture in Egypt has become an increasingly important activity, as an immediate source of animal protein required for the country's growing population. The total fish production in 1998 was estimated at 546 000 t, of which 26% is from aquaculture. Most fish farms practice polyculture where tilapia represents about 38% of the total production. Along with tilapia (*O. niloticus* and *O. aureus*), mullets and carps are also stocked. The total production of tilapia fry from hatcheries or fish farms in 1998 was estimated at about 49.9 million fry. *O. niloticus* and *O. aureus* dominate the fry production. Most consumers in Egypt prefer tilapia compared with other freshwater fish.

There are four tilapia species in Egypt: Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*), white tilapia (*S. galilaeus*) and redbelly tilapia (*T. zillii*) (ElGhobashy,

2001). The most classification methods of tilapiine species is based on reproduction, development, feeding, structural characteristics and biogeography (Trewavas, 1983). However, such characters had limited value for identification purposes because they show considerable interpopulation variation and differences between species are small (Abban, 1988). Protein electrophoresis has been extensively used to discriminate species of tilapia (McAndrew and Majumdar, 1984) and their hybrids (Macaranas *et al.*, 1986) but this technique could not discriminate subspecies of *O. niloticus* (Seyoum, 1990). Mitochondrial DNA markers have been used successfully to identify the subspecies of *O. niloticus* (Seyoum and Kornfield, 1992) but little effort has yet been devoted to the analysis of nuclear DNA for this purpose.

By detecting genetic variation, genetic markers may provide useful information at different levels: population structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness (Feral, 2002). The polymorphic DNA markers that were shown to genetically link to a trait of interest could be used for individual and pedigree identification, pathogenic diagnostics, and trait improvement in genetics and breeding programmes. Morphological criteria (Bernardi and Talley 2000), biochemical data (Jensen 2000). Yoon and Kim, (2001) used randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) to study the genetic similarity and diversity of cultured catfish *Silurus asotus* populations collected from two areas in western Korea Asensio *et al.*, (2002) found that RAPD analysis produced clear fingerprints from which the three fish species: Grouper, (*Epinephelus guaza*) , Wreck fish (*Polyprion americanus*) and Nile perch (*Lates niloticus*) could be easily identified. This approach is rapid and reliable and offers the potential to detect fraudulent or unintentional mislabeling of these species in routine seafood authentication analysis.

The purpose of this study was to employ the RAPD method to uncover sufficient informations and detect nuclear DNA variations in the three genera of tilapia fishes: *Tilapia, Oreochromis* and *Sarotherodon*; and to identify the RAPD variation in two *Oreochromis* species (*O. aureus* and *O. niloticus*).

MATERIALS AND METHODS

The present study was carried out at the Nucleic Acid Research Dept., (GEBRI), Mubarak City For Scientific Research & Technology Applications, and Animal and Fish Production Department, Faculty of Agriculture, Saba-Bacha, Alexandria University, Alexandria, Egypt.

Genera and species studied:

The genera and species studied are presented in table (1).

[illegible] and suspended in 500 µl STE (0.1 *M* NaCl, 0.05 *M* Tris and 0.01 *M* EDTA, pH 8). After adding 30 µl SDS (10 per cent) and 30 µl proteinase K (10 mg ml), the mixture was incubated at 50°C for 30 min. DNA was purified by successive extraction with phenol, phenol : chloroform: isoamyl alcohol (25:24:1) and chloroform : isoamyl alcohol (24:1) respectively. DNA was precipitated with ice-cold absolute ethanol and washed with 70 per cent ethanol. The pellet was dried and resuspended in 200 µl mill Q water.

RAPD analysis:

The polymerase chain reaction mixture (25 µL) consisted of 0.8 U of Taq DNA polymerase (enzyme), 25 pmol dNTPs, and 25 pmol of random primer, 2.5 µl. 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermocycler (Perkin Elmer 9700). The PCR programme included an initial denaturation step at 94°C for 2 minutes followed by 45 cycles with 94°C for 30 seconds for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72 °C for 10 minutes were carried out. The samples were cooled at 4°C. The amplified DNA fragments were electrophoretically separated on 2.5% agarose gel and stained with ethidium bromide. ΦX174 DNA marker (bp 1353, 1078, 872,....., 72) was used in this study. Each amplified pattern was visualized on a UV transilluminator and photographed.

Random Amplified Polymorphic DNA (RAPD) was carried out using the random primers presented in table (2).

Table (2): The sequences of the primers used and their annealing temperatures.

Primer	Sequence 5`- 3`	Annealing Tm/Sec
1	AGG CCC CTG T	34 / 30
2	ATG CCC CTG T	
3	AAA GCT GCG G	
4	ACC GCC GAA G	
5	ACC GGG AAC G	46/ 30
6	AGC AGG TGG A	
7	CAG GCC CTT CCA GCA CCC AC	52/ 30
8	GAA ACG GGT GGT GAT CGC AG	
9	GGT GAC GCA GGG GTA ACG CC	54/ 30
10	GGA CTG GAG TGT GAT CGC AG	52/ 30
11	GGA CTG GAG TGG TGA CGC AG	
12	GAA TGC GAC G	46/ 30
13	ATG ACG TTG A	
14	CTG AGG AGT G	
15	GGG CTA GGG T	
16	GGC ACT GAG G	
17	CGC TGT CGC C	
18	AGT CCT CGC C	
19	TGG TGG ACC A	
20	AGC CAG CGA A	34/30

RESULTS AND DISCUSSION

An evaluation of 20 different primers used in this study (table 2), was carried out. Five primers are 20 mer and 15 are 10 mer. All the primers were examined, 19 (95%) of them produced different RAPD fragment patterns (tables 3 and 4) but, only 1 (5%) primer (10 mer) resulted in no product.

Data presented in table (3) and fig. (1) showed that total number of amplification products and polymorphic fragments obtained from a survey of 20 primers of random sequence among the 3 genera of tilapia fish (*Tilapia, Sarotherodon, Oreochromis*). Sixteen of 19 primers (84.21%) gave polymorphic bands among the three genera, however three of them (15.79%) produced no polymorphism. The total number of fragments generated per primer varied between 3-15. On the other hand, the number of polymorphic fragments per primer ranged from 2 to 4.

The obtained results are in agreement with the findings of several authors in their researches (Bernardi and Talley 2000; Jensen, 2000; Feral, 2002 and Ali *et al.*, 2002).

Table (3) : Summary of the number and characteristics of amplification products obtained from a survey of 20 primers of random sequence among three genera of tilapia fish.

Primers no.	No. total bands	No. polymorphic bands	Size range bp
1	6	3	872-281
2	6	2	872-281
3	9	0.0	310-194
4	11	4	872-234
5	14	4	1078-194
6	6	0.0	872-234
7	9	0.0	872-234
8	6	2	603-234
9	8	3	872-281
10	10	3	872-234
11	14	2	1353-234
12	6	2	872-234
13	15	4	1078-234
14	10	4	603-234
15	13	2	872-234
16	5	2	603-194
17	11	2	872-194
18	6	2	1078-234
19	3	2	310-194
20	0.0	0.0	0.0

The genetic variations among the three genera may be due to differences in growth rate, fertility, high salinity tolerance, low temperature tolerance and phenotype of each genus. Moreover, about 70 species of tilapia have been subdivided into three genera: *Orechromis* for species that are only maternal mouthbrooder and with distinctive and conspicuous breeding coloured males, *Sarotherodon* for mouthbrooder but the eggs and embryos are brooded in the mouth of both parents and *Tilapia* for substrate spawners that guard their developing eggs and larvae, either by fanning of females are coping with intruders by males (Trewavas, 1983).

In respect to molecular differences between the two species of tilapia (*O. aureus* and *O. niloticus*), results in table (4) and fig. 1 (lanes 3 and 4) showed the total number of amplified products and polymorphic fragments obtained from a survey of 19 primers of random sequence. All of 19 primers (100%) gave polymorphic bands between the two species. The total number of fragments generated per primer ranged from 3-13. On the other hand, the number of polymorphic fragments per primer varied between 1 -6. It can be observed that primer no. 13 have specific band at size of 1078 bp, this band was found in *O niloticus* only (fig 1; C; lane 4) It can used this band to discriminate *O niloticus* from the other species Also, it can be observed that RAPD analysis produced

clear fingerprints from which the two species could be easily identified and this variation may be due to differences in growth, cold tolerance and phenotype of each species (Sarig, 1969), moreover resulted from difference at the level of DNA molecule between them. Furthermore, the sensitivity of RAPD technique played a good role in detection of these differences. This observation agrees with Capili, (1990), Seyoum and Kornfield (1992), and Bardakci and Skibinski (1994), they suggested that RAPD analysis might be more sensitive than other molecular techniques like mtDNA analysis which has failed to reveal variations within tilapia population. In common with other molecular techniques, RAPD analysis has been successful in identifying markers, which distinguish tilapia species. Random amplified polymorphic DNA (RAPD): Short arbitrary sequences of nucleotides can be used to amplify random fragments of DNA. This technique produces a large number of fragments, many of which are individual-specific (Welsh and McClelland, 1990; Williams *et al.*, 1990). If inheritance is verified, RAPD patterns can be also used for population genetics (Williams *et al.*, 1990). However, there is a possibility of dominance of RAPD markers that may hide genetic variation and the smaller fragments are usually not visualized. They can be well suited for the characterization of breeding systems or the calculation of genetic parameters, or phylogenetic inferences (Grosberg *et al.*, 1996).

Table (4): Summary of the number and characteristics of amplification products obtained from a survey of 20 primers of random sequence between two species of tilapia fish (*O. aureus* and *O. niloticus*).

Primers no.	No. total bands	No. polymorphic bands	Size range bp
1	5	2	872-281
2	6	2	310-281
3	4	2	310-194
4	4	2	872-234
5	13	3	1078-194
6	7	3	603-234
7	3	3	872-234
8	3	1	310-234
9	6	6	872-281
10	5	5	872-234
11	10	4	1353-234
12	5	3	872-234
13	8	5	1078-234
14	4	4	603-234
15	5	5	872-234
16	4	2	310-194
17	9	3	872-194
18	5	3	872-234
19	3	1	310-194
20	0.0	0.0	0.0

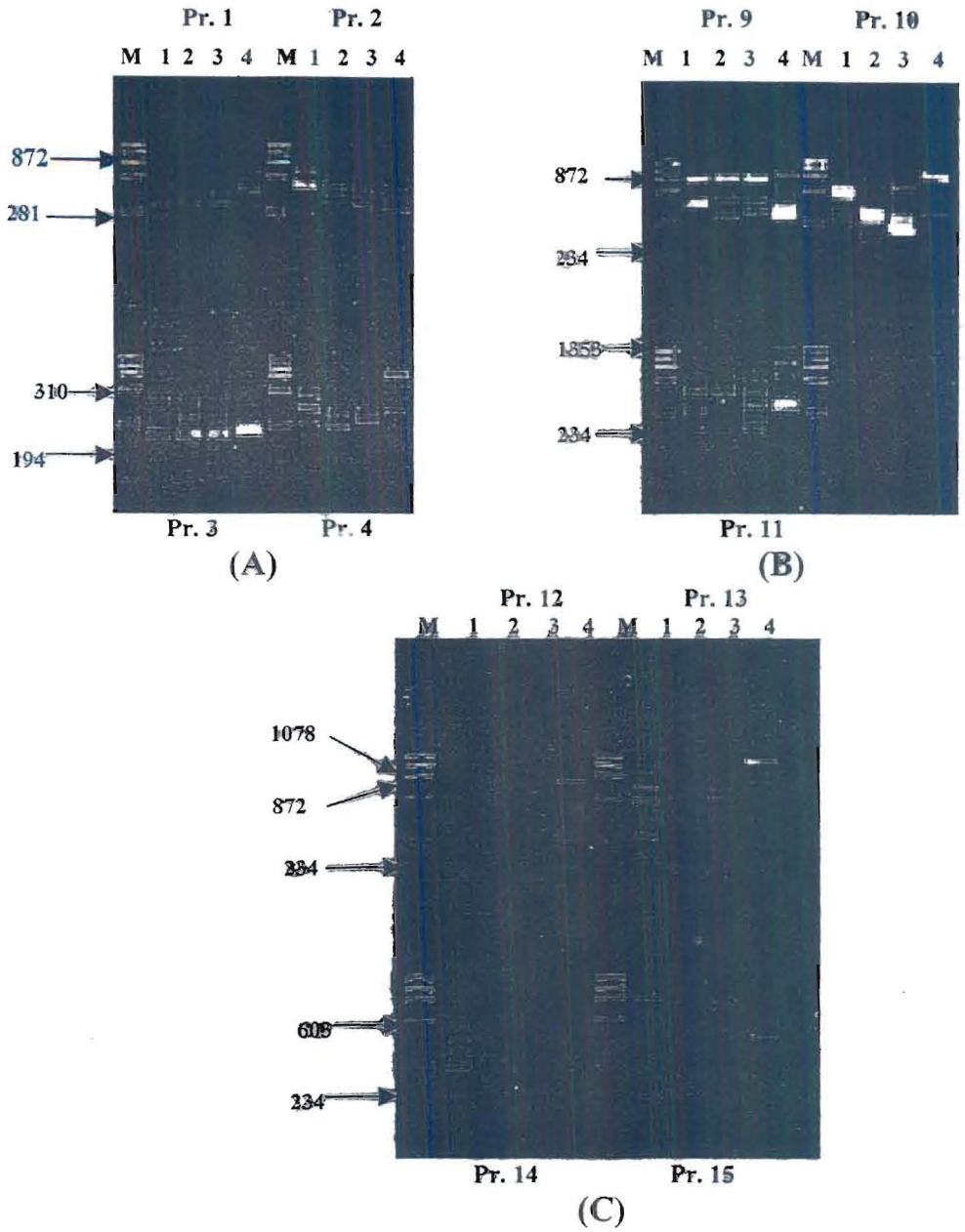

Figure (1): RAPD amplification products generated from three genera of Tilapia by random: A) Primers (1, 2, 3,and 4). B) Primers (9, 10 and 11). C) Primers (12,13,14 and 15). ; The 5 lanes of each primer are:
- M is DNA marker - Lane 1 is *Tilapia* genus - Lane 2 is *Sarotherodon* genus -Lanes 3 and 4 are *O. aureus* and *O. niloticus*, respectively.

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