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RAPD-PCR for DNA-Fingerprinting of Egyptian tilapia

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Abstract: Evaluate of common patterns of genetic variations or similarities among three species of tillapine through the DNA fingerprinting analysis was performed in this study using RAPD-PCR (Random amplified polymerase chain reaction) using 10 arbitrary primers, out of 10 primers used, 7 primers gave strong sharp distinct bands. The 7 primers produced a total of 15 , 13 , 16 , 14 , 18, 14 and 17 bands respectively. Depending on the similarity coefficient through the used primers, the similarity between *Oreochromis niloticus and Oreochromis galilaius* was 95% and between *Oreochromis niloticus* and *Tilapia zilli* was 80% and between *Oreochromis galilaius* and *Tilapia zilli* was 75%. The values of the genetic distances obtained were utilized to generate a distance matrix to construct a dendrogram which linked the studied species. The results of DNA fingerprinting of the studied fishes can be taken into consideration as a joint patterns of similarity and probability of Hybridization between the very closed species to improve the genetic characters. [New York Science Journal. 2009;2(2):20-25]. (ISSN: 1554-0200).

Keywords: RAPD-PCR, DNA-Fingerprinting, Egyptian tilapia

Introduction

Morphological studies have been especially successful in defining species and in organizing these species into genera. These groupings have usually been confirmed when examined with molecular approaches. Molecular characters have revealed some cryptic species (**Avise, 1994)** and identified some incorrectly split groups (e.g., species inclinid klep fish genus *Gibbonisia* by **Stepien and Rosenblah, 1991**).

Although morphological studies have generally been successful in defining genera, it is rare to find studies which present a hypothesis of relationship above the level of species comprising a genus, primarily due to a lack of congruence of characters **(Stepien and Kocher, 1997),** fortunately this is one of the strengths of molecular data, and inter- and intrageneric relationships are now being rapidly tested and elucidated. Molecular data are also means used to assess the phylogenetic relationships among populations.

Development in RAPD-PCR technology is known as Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). This approach of DNA polymorphism which based on PCR amplification of DNA segment using single primers of arbitrary nucleotide sequences has been developed by **Williams *et al.* (1990)** and **Welsh and McClelland (1990).** This technique involves amplification of certain regions of the nuclear genome flanked by inverted sequences complementary to particular nucleotide 10-mer primer, provided that the primer anneals within a range of annealing temperatures (35- 40°C). The primer randomly anneals to an unknown segment on one of the DNA strands. When two species, strains, or individuals are compared, polymorphism between them will be revealed on agarose electrophoresis gels by presence or absence of an amplification product., this method has been applied to the discovery of genetic

markers for mapping studies (**Poslethwait *et al.*, 1994**) and to elucidate the phylogenetic relationships between species (**Bardakci and Skibinski, 1992; Welsh and McClelland, 1990) . In this investigation , RAPD-PCR was used to examine the DNA-fingerprint and the similarity among three tilapia fish in Egypt, *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zilli* .**

Materials and Methods

Fish samples:

Fish samples of three species *Oreochromis niloticus, Sarotherodon galilaeus* and *Tilapia zillii* were collected from El-Abbassa fish farm , from October to December 2005

DNA extraction :-

Genomic DNA was extracted using the phenol- chloroform extraction method **(dinesh et al., 1993).**

Polymerase chain reaction (PCR) amplification was carried out following the RAPD- fingerprinting protocol previously established for fish **(Dinesh et al . , 1993) .** Genomic DNA was amplified in reaction volume of 50 µl using a 10 –mer seven RAPD primers with G + C content 60 and 70 %.

the sequences of primers were : **GAAACGGGTG , AATCGGGCTG , GGGTAACGCC, CAGCACCCAC, GTGATCGCAG, CCGGGAATCG and AGTCAGCCAC**

The reaction mixture was prepared using 2.5 *ml* of 10 X PCR buffer , 2.5 ml 50 mM Mgcl2 , 1 ml 10 mM DNTPs , 1 – 7 ml primer , 2 ml DNA (100 - 200 ng) 0.5 ml Taq DNA polymerase enzyme and H 2O in a total volume of 50 ml. Amplification was carried out with the following cycle program: 4 min. of denaturation at 94ºc; 37cycles of amplification at 94ºc (1 min); 36ºc-42 ºc (2 min) and 72 ºc (1 min). Soaking was carried out in 4ºc.

Analysis of RAPD - PCR products:

The products of amplification were analyzed by electrophoresis on

1.4% agarose gels in 1X TBE buffer, visualized under UV trans-illuminator and photographed by a Polaroid camera.

(1)Genetic statistical analysis:-

The similarity coefficients between the three tilapia species were calculated based on pair wise comparison between them for primers using the formula:

$$\delta = 2\, N_{xy} / (N_x + N_y)$$

Where Nx and Ny are the number of bands in individuals X and Y.

N_{xy} is the number of shared bands (**Nei and Li, 1979; Lynch and Milligan , 1994**) .

The average pair wise similarity (S) was then calculated as an average across primers. The similarity values were converted into genetic distance using the formula: D = 1 - S (N**ei and Li, 1979).**

The data derived from this formula was plotted to establish a matrix of distance using computer program of unweighted pair- group Arithmetic average cluster analysis to construct a dendrogram for the three tilapia species.

Results

Out of 10 primers used, 7 primers gave strong sharp distinct bands. The RAPD fingerprints of three fish species are shown in fig.(1) the total bands generated by the primers A-G respectively are: 15 , 13 , 16 , 14 , 18, 14 and 17 bands respectively.

Figure1(A-G) have four lanes, from left to right are: the DNA marker, *Oreochromis niloticus, Sarotherodon galilaeus, and Tilapia zilli* PCR products.

Using primer A, the amplification products of *Oreochromis niloticus* DNA by application of RAPD technique showed five bands of molecular weights 207, 328, 569, 790 and 1145 bp. While *Sarotherodon galilaeas* produced 5 bands of molecular weights 207, 383, 472, 734 and 1003 bp. and *Tilapia zilli* showed five bands, 207, 362, 461, 764 and 1040 bp.

(Fig.1-A)

The RAPD-PCR products using primer B revealed six bands in *Oreochromis niloticus*, 253, 317, 412, 506, 758 and 1017 bp. And three bands only in *Sarotherodon galilaeus*, 506,758 and 1029 bp. But in *Tilapia zilli* it revealed four bands of molecular weights 390, 506, 1029 and 1425 bp.(Fig.1-B)

In primer C, *Oreochromis niloticus* produced five bands, 202,403, 581, 632 and 1403 bp., where in *Sarotherodon galilaeus* five bands were appeared as 217,418,571,632 and 1406 bp. and six bands appeared in the amplification of *Tilapia zilli* DNA, 92, 209,461,581,632 and 1403 bp. .(Fig.1-C).

Primer D revealed six bands of molecular weights 196, 270, 449, 578, 810 and 901 bp. in case of *Oreochromis niloticus*, but produced four bands of molecular weights 254, 449, 578 and 810 bp. in case of *Sarotherodon galilaeus* and four bands in Tilapia *zilli* of molecular weights 578, 710, 810 and 930 bp. .(Fig.1-D). The amplification products of RAPD-PCR using primer E produced eight bands utilizing *Oreochromis niloticus* DNA, there molecular weights were 67,123, 215, 278, 340, 461, 720and 903bp. and six bands in *Sarotherodon galilaeus* of molecular weights 59, 187, 270, 390, 607 and 840 bp. Also it revealed four bands in Tilapia *zilli*, 117,270, 390 and 607 bp. .(Fig.1-E). Primer F produced six bands in the amplification of *Oreochromis niloticus* DNA, there molecular weights were 168, 173, 288, 390, 582 and 783bp.; four bands of molecular weights 317, 405, 591 and 698 bp. in *Sarotherodon galilaeus*, while in Tilapia *zilli* it evealed four bands of molecular weights 317, 405, 591 and 677 bp. .(Fig.1-F). Finally using primer 7, six bands appeared in *Oreochromis niloticus*, there molecular weights were 170, 202, 460, 591, 632 and 1343 bp. ; five bands in case of *Sarotherodon galilaeus* of molecular weights 270, 431, 583, 620 and 1281 bp. and six bands of molecular weights146, 270, 436, 583, 620 and 281 bp in Tilapia *zilli*. .(Fig.1-G)

Phylogenetic relationships:

Depending on the data produced from RAPD-PCR amplification and fish DNA fingerprinting, similarity coefficient was obtained from statistical analysis to assess the similarity between the three tilapia fish species. A

linkage map (dendrogram) is constructed among the three tilapia fish species of (Fig.2).

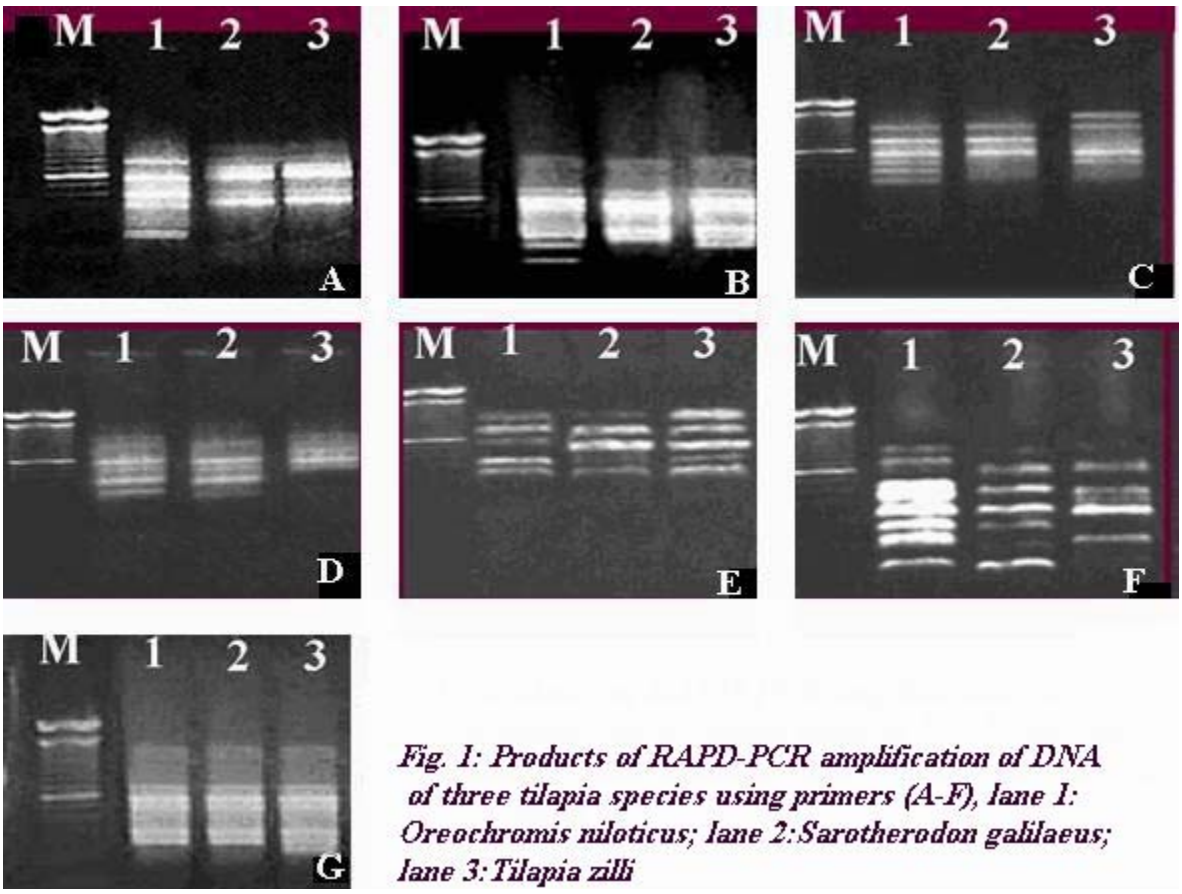

Fig. 1: Products of RAPD-PCR amplification of DNA of three tilapia species using primers (A-F), lane 1: Oreochromis niloticus; lane 2:Sarotherodon galilaeus; lane 3:Tilapia zilli

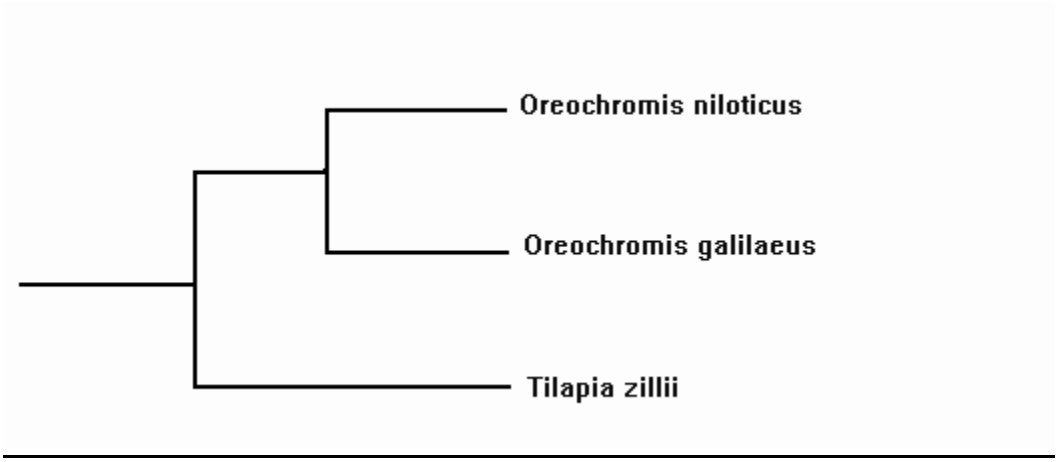

Fig.2: Dendrogram generated by clustering using Unweighted Pair-Group Arithmatic Avarage analysis of 1-s values (based on Nei and Li's index) computed from pairwise comparison between three tillapine fish speci

Discussion

RAPD bands in this study were always variant (i.e., strong, faint, fuzzy and sharp bands) generated with each primer because one or more copies of DNA may exist per genome or may be attributed to the varying of the annealing process between the primer and the DNA. This problem of mixed bands shows the well known sensitivity of PCRs **(Bielawski *et al*., 1995).** RAPD fragments generated by primer four produced low polymorphism among fishes studied. This primer sequences may have annealed to variable sequences, which might be of great utility at lower taxonomic levels, e.g. for the differentiation of very related species. However, in RAPD fragments generated by the other primers, there were high degree of polymorphism, their sequences may be considered as more conserved sequences, which are most useful in higher taxonomic levels and evolutionary relationships. These results are in agreement with **Bardkci and Skibinski (1994)** who stated that, patterns of similarities and differences between populations showed broad agreement across primers and the overall similarity level varied between primers. Thus, primer choosing is a very important for this technique.

RAPD fingerprinting has been used to construct a genetic linkage map **(Postlewait *et al*., 1994).** In this study, three species of tilapia fish were studied genetically through the RAPD technique to put a species fingerprint and to identify the similarity coefficient among the fishes under study. This coefficient represents a measure of the shared bands two or more different species within the same, and different, primers .These are important measurements that help to quantify the degree of relationships between different species.

The description of this similarity coefficients is not simple, especially when more than one character are involved in the same cluster. Thus, *O. niloticus* and *S. galilaeus* are found to have a similarity coeffecient of 95%, where between *O. niloticus* and *T. zillii* is 80% and between *S. galilaeus* and *T. zillii* is 75%. The genetic similarity between *O. niloticus* and *S. galilaeus* was higher compared with that between *O. niloticus* and *T. zillii*. Also, between *S. galilaeus* and *T. zillii* there was low similarity compared with that of *O. niloticus*. The dendrogram (fig. 2) indicates the relationship among the three *tilapia* species which are of family *Cichlidae* , taking into consideration the close relationship between *O. niloticus* and *S. galilaeus*

However, this study concluded that the very high similarity between *O. niloticus* and *S. galilaeus* leads to high probability of hyberidization between them, but not between *O. niloticus* and *T. zillii.* These findings are an indication of the distinct character of *T. zillii* territoriality, in contrast to the other two species, in spite of the close morphology of body shape they all share.

It can be concluded also that, RAPD-PCR could prove to be a useful tool for estimating the genetic variability and degree of similarity among fish species.

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