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Genetic status of tilapia at Badore landing site using RAPD markers

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Abstract

Molecular technique based on Random Amplified Polymorphic DNA (RAPD) analysis was applied to study genetic status among tilapia species from Badore landing site of Lekki lagoon. Individual variations within species' population were assessed using PCR-RAPD analysis with five Operon primers (OPC 04, OPA02, OPB08, OPE02 and OPF03, Operon Technologies Inc. USA) which revealed different banding patterns of varying primer reproducibility. Graphical representation using UPGMA cluster analysis produced a dendrogram chart with five clusters (θ, π, ε, ∑ and Ω) indicating different degrees of variations and similarities. There were various levels of genetic similarity observed possibly due to hybridization. Nevertheless; few distinct variations among the samples were visible, showing possible genetic variability. At 0.89 (89%) coefficient, cluster, θ is made up of 7 samples which are genetically similar. At 0.834 (83.4 %) coefficient, distinct sample BT12 forms a cluster (π) with cluster θ which shows they are related at this coefficient. Cluster Ω (84 % coefficient) comprising of 7 samples forms another cluster with a distinct sample BT06 at about 0.79 coefficients. At 78.6% coefficient (cluster ε). All the samples are genetically similar except sample BT17. This distinct sample can increase genetic variability by a cross between it and other strains of tilapia. Therefore, care should be taken by fish farmers who buy or use the fish samples from this landing site for culture. Proper molecular characterization of this fish species before culture becomes necessary to avoid genetic problems.

Keyword: *Genetic, Tilapia, RAPD, Badore.*

Introduction

Tilapia belongs to the family Cichlidae, a family of great diversity. Conservative estimate of the number of species of cichlids is 1,300 or more (Colman, 2001). It is native to tropical Africa and Palestine but has spread to various continents of the world. They differ greatly in size and taxonomic group (Olojo, 2003). Essentially there are three genera of tilapia, based on reproductive behaviour. They are *Oreochromis, Sarotherodon* and *Tilapia* (Christian, 2002). According to the author, *Oreochromis,* species are maternal mouth brooders, *Sarotherodon* species are bi-paternal mouthbrooders while Tilapia are substrate spawners. Tilapias are commercially important fish in rural communities like Badore village located along Lekki lagoon system of Lagos state.

Taxonomic, ecological and evolutionary research questions have been addressed using current advances in molecular biology which serve as novel tools in characterizing fish including tilapia. Differences in DNA sequence can be observed with a level of accuracy that was previously impossible. The bulk of variation at the nucleotide level is often not noticeable at the phenotypic level (Gupta et al., 2008). Random Amplified Polymorphic DNA (RAPD) marker's use, which identifies DNA polymorphisms, is PCR based. It is relatively cheap, reliable and offers the potential to detect fraudulent or unintentional mislabeling of fish species in routine food authentication analysis (Maldini et al., 2006). Segments of genomic DNA can be amplified in a polymerase chain reaction (PCR) with random oligodeoxynucleotides as primers (Williams et al., 1990) followed by Agarose gel electrophoresis. RAPD markers are usually dominant and polymorphic and are observed as the presence or the absence of DNA bands of a particular size, the author reported. RAPD has been used to estimate genetic diversity of fish populations (Lopera-Barrero et al., 2006; Brahmane et al., 2006;Fouz et al., 2007). However, shortcomings of RAPD markers include the difficulty of demonstrating Mendelian inheritance of the loci and the inability to distinguish between homozygotes and heterozygotes (Liu and Corde, 2004).Therefore, this research is a preliminary investigation on genetic stautus of Tilapia species in Badore landing site using molecular techniques.

Materials and Methods

Seventeen specimens of varying species of the genus, *Tilapia* were obtained from landing site of Badore , Lagos state. The project was conducted at Biotechnology laboratory of Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos. Genomic DNA was isolated using salting out method from caudal fin according to Lopera-Barrero et al. (2008). Caudal fins (approximately 3.5-4.5 g) of *Tilapia species* collected were placed in Eppendorf microtubes with 90% absolute ethanol and maintained in a freezer at -20°C. Lysis buffer (550 µl) containing 7 µl of 200 μgml^{-1} of proteinase K was added. The samples were incubated in water bath at 50°C for 12 hrs. Sodium chloride (660 µl of 5 M) was added and centrifuged at 12,000 rpm for 10 mins. The supernatant was transferred to a new tube and 700 µl of cold absolute ethanol was used to precipitate the DNA. The samples were incubated at -20°C for 2 hrs. The DNA sample was centrifuged and washed with 700 µl of 70 % v/v ethanol. The sample was resuspended in 80 µl TE buffer (10 mM of Tris pH 8.0 and 1 mM of EDTA). Ribonuclease (30 μgml^{-1}) was added and incubated in water bath at 42°C for 4 hours. This was stored in the freezer at – 20 °C until it is ready to be used. The polymerase chain reaction mixtures (25 µl) consisted of 0.125 µl of Taq DNA polymerase (1.25 U) (Fanzyme), 0.5 µl dNTPs, 0.2 µl primers of RAPD (100 $pmol\mu l^{-1}$), 2.5 µl. 10 X Taq DNA polymerase buffer, 5 µl of genomic DNA and 15.73 µl distilled water.

The PCR amplification was performed in a Biorad I- cycler programmed for initial denaturation step at 94°C for 5 minutes followed by 45 cycles of denaturation at 94°C for 60 seconds, annealing at 45°C for 45 seconds and extension at 72°C for 1min.30secs; a final extension at 72°C for 5 minutes was carried out. The samples were cooled to 4°C. The amplified DNA fragments were separated on 1.5 % (w/v) agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator and photographed. Each RAPD assay was done three times to ensure reproducibility. RAPD primers used were from Operon technology, USA (Table 1).

Table 1: Sequences of primers used in the amplifications with RAPDs

RAPD primer	Primer sequence	Source
OPC 04	CCGCATCTAC	Operon technology USA)
OPA 02	TGCCGAGCTG	Operon technology(USA)
OPB 08	GTCCACACGG	Operon technology(USA)
OPF 03	CCTGATCACC	Operon technology(USA)
OPE 02	GGTGCGGGAA	Operon technology(USA)

Results and Discussion

With the **5** primers used, 3 showed both stable amplification and polymorphism while 2 have no reproducibility. Phylogenetic analysis using PASTA - PAlaeontological STatistics, ver. 1.89 software produced a dendrogram with 16 clusters at various degrees of similarities coefficient (Figure 1). Five major visible clusters (θ, π, ε, ∑ and Ω) were observed in the dendrogram at various degrees of coefficients. At 0.89 (89%) coefficient, cluster θ is made up of 7 samples which are genetically similar. At 0.834 (83.4 %) coefficient, distinct sample BT12 formed a cluster (π) with cluster 0 which shows they are related at this coefficient. Cluster Ω (84 % coefficient) comprising of 7 samples formed another cluster with a distinct sample BT06 at 0.79 coefficients .This also indicates that the seven samples have high genetic similarity but not with BT06 until at 79% coefficient. The entire samples were genetically similar at 78.6% coefficient (cluster ε) except samples BT17. High levels of genetic similarity were visible among the samples in the population but not with samples BT12, BT06 and BT17. Furthermore, genetic similarity between the entire population and samples BT17 were very low. This result can be used in assessing genetic relationship among these fish. This is in agreement with Palti et al. (1997) who reported that DNA fingerprints of mixed DNA samples can be useful in assessing relationship between closely related populations due to the high level genetic differentiation detected.

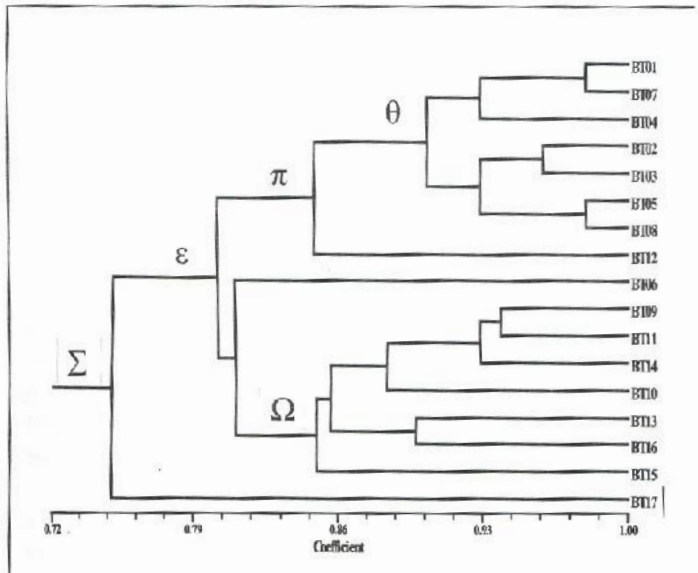

Fig. 1: Dendrogram depicting the degree of relationship among Tilapia species from Badore landing site.

The coefficient represents a measure of the shared bands of two or more different fishes within the same genus, *Tilapia*, with varying primers. These important measurements may help to quantify the degree of relationships between different tilapia and provide information on closeness within the same genus where variant occurs. High similarity among this tilapia under study can lead to increased level of hybridization, but not with sample BT17. This particular strain can increase genetic variability by a cross between it and other strain of tilapia.

The present study provide evidence that the RAPD markers can be effectively used to discriminate among

cichlid populations. This agrees with that obtained by many workers such as Bardakei and Shibinski (1994) who found out that RAPD has been used successfully to detect variations among populations, sub-populations and species. As a result of hybridization which occurs among tilapia in the wild, many of these tilapias are morphologically similar. RAPD methodology has therefore been applied in studies aiming at species identification whose morphological discrimination remains difficult (Takagi and Taniguchi, 1995; Dinesh et al., 1995; Almeida et al., 2001). RAPD-PCR have again shown to be a valuable tool for estimating the degree of genetic variation among fish species as has been reported by other workers (Palti et al. 1997; Barman et al., 2003; Jayasankar et al., 2004; Lopera–Barrero et al., 2006; Shair et al., 2011;Mojekwu et al., 2013).

Conclusion

In conclusion, Tilapia species landed at the landing site are genetically similar with the exception of BT17. This strain can encourage genetic variability, though it represent only about 1% of the sample studied. The level of similarity may be attributable to high level of hybridization that takes place in the wild among the genus, Tilapia. It is therefore advisable to carry out proper screening using appropriate molecular techniques before fish farmer use this stock on their farms.

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