ABG -35

Evaluation of Single Nucleotide Polymorphisms and Genetic Diversity of Tilapia Fish Using Mitochondrial D-Loop and Cyt-B Regions

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Abstract
The research was aimed at analysing single nucleotide polymorphisms (SNPs) and genetic diversity on D-loop and Cyt-b regions of the mitochondrial DNA of tilapia fish. Fifteen and thirteen tilapia fish were obtained from two populations, South South (Domita farm) and South West (Odeda farm), Nigeria. DNA extraction was performed using Quick-gDNA™ mini Prep kit followed by PCR and sequencing of the two mtDNA regions. Statistical analyses were carried out on the aligned sequenced data using MEGA version 6.06, Dnasp 5.1, Codon code aligner 6.06 as well as NETWORK 4.6.1.1. MtDNA polymorphism was highest in the D-loop of South-South population with 176 polymorphic sites, while South-West population had 162 polymorphic sites translating to 176, 162 and 144 SNPs with higher non-synonymous substitutions than synonymous substitutions. Haplotype diversities (Hd) were 1.00 ± 0.024 and 1.00 ± 0.030 while nucleotide diversities were 0.168 ± 0.086 and 0.161 ± 0.084 for D-loop of South-South and South-West populations, respectively. For Cyt b region, haplotype and nucleotide diversities were 0.91 ± 0.003 and 0.051 ± 0.016. Positive selection was more on mtDNA D-loop of tilapia sampled from South South than those from the South Westas well as Cyt-b region of tilapia fish from South South, while demographic expansion was not significant. Higher SNPs were revealed in mtDNA D-loop when compared with mtDNACyt-b region of the tilapia fish.
Keywords: SNPs; Genetic diversity; mtDNA D-loop; Cyt-b and Tilapia fish

Introduction

Tilapia fish is the most widely harvested and consumed fish after carp globally [FAO, 2009]. Commercially, tilapia fish is highly consumed with a global harvest of approximately, 4,677,613 tonnes in 2013 [Fitzsimmons *et al.,* 2014]. For emphasis, China is the world's largest producer of tilapia with about 1,600,000 tonnes production, while in Africa; Egypt is the largest producer of about 800,000 tones [Fitzsimmons *et al.,* 2014]. Nigeria has an estimated population of over 160 million with a coastline measuring approximately 853 kilometres. This vast coastline according to [Mjoun and Kurt, 2010] can be harnessed for tilapia fish farming, which might probably have the capacity to make a significant contribution to agriculture.

Tilapia fish has received little or no research attention comparatively, its importance as rich protein, potassium, phosphorus, vitamin B12 as well as low-fat fat content (Job *et al.,* 2015; Mjoun and Kurt, 2010) notwithstanding. The other frightening important issue is the genetic erosion in this species of fish orchestrated by indiscriminate and over-exploitation from the wild by fishermen in the bid to bridging the demand-supply gap. The implication therefore, is that if efforts are not intensified, especially in research geared towards domesticating, conserving and integrating tilapia farming into the agricultural programme in Nigeria, it might spell doom.DNA sequenced data have been reported to be very informative in explaining genetic relatedness among species of organisms. Out of the two major genomic DNAs – nuclear and mitochondrial, mitochondrial DNA (mtDNA) have been used in studying stock structures in several vertebrates (Shanker *et al.,* 2004; Zink *et al*., 2000; Castro *et al*., 1988).

There are several landmarks on the DNA that can be utilized for the identification and characterization of species of organisms, especially Single Nucleotide Polymorphisms (SNPs). SNP-based research is geared towards studying the genetic differences between species for the prediction of phenotypes and phylogeny. Giving that when SNPs occur inside a gene, they create different variants or alleles of that gene and the sequences tend to be transmitted unchanged across generations; in this present study, using SNPs markers on D-loop and cyt-b regions of mtDNA, genetic diversity, polymorphisms and haplotype in tilapia fish sampled from two populations in Nigeria were investigated.

Results and Discussion

The accession numbers for mtDNA D-loop sequence for south-south and South West tilapia population are MF385001 and MF385002 while Cyt b sequence for south south tilapia population is MF384326 for your reference. The total number of the aligned site was 745 for D-loop and 1022 for Cyt-b region. mtDNA polymorphism was highest in the D-loop of South-South population with 176 polymorphic sites, while South-West population had 162 polymorphic sites. The lowest polymorphism was observed in Cyt-b of South-South population with 144 polymorphic sites (Table 1). The mtDNA D-loop of tilapia population from South West revealed 176 SNPs resulting in 155 non-synonymous and 21 synonymous mutations while mtDNA D-loop of tilapia population from South South revealed 162 SNPs, which resulted in 148 non-synonymous and 14 synonymous mutations, respectively (Table 2). From the mtDNAcyt- b sequence of the South-South tilapia population, 144 SNPs were detected resulting in 137 non-synonymous and 7 synonymous mutations (Table 3). Selection analysis revealed positive and negative selection in the populations. The median joining network analysis revealed interesting result as haplotype 1-15 associated with D-loop of South-South samples were clustered together while haplotype 16-28 associated with D-loop of South-West samples had two groups. Similarly, 9 haplotypes were identified from 15 samples sequenced for mtDNAcyb-b of tilapia samples from S/S population. Tajima's D values were -1.080, -0.83, and 0.673, while the Fu's Fs values were -1.681, -1.206, and 0.584 for samples in S-S D-loop, S-W D-loop and S-S cyt-b, respectively.

Table 1: Genetic diversity parameters among tilapia fish from the studied populations

Diversity indices	SS D-loop	SW D-loop	SS Cyt b
Number of sequences	15	13	15
Number of sites	745	745	1022
Monomorphic sites	568	574	878
Polymorphic sites	176	162	144
Singleton variable site	77	38	29
Parsimony information site	99	124	112
Number of haplotypes	15	13	9
Haplotype diversity (Hd)	1.00 ± 0.024	1.00 ± 0.030	0.91 ± 0.003
Nucleotide diversity (π)	0.168 ± 0.086	0.161 ± 0.084	0.051 ± 0.016
Average number of pairewise differences	52.07	49.14	52.114
Sequence conservation	0.763(76.3%)	0.780(78%)	0.859 (85.9%)
Minimum number of recombination	42	18	1
Tajima's D	-1.080(p>0.10)	-0.83(p>0.10)	0.673(0.01)
Fu's F	-1.681(p>0.10)	-1.206 (p>0.10)	0.584 (p>0.01)

Table 2: Mutation analysis of single nucleotide polymorphism (SNPs) in mtDNA D-loop of tilapia fish from south south and south west, Nigeria

S/N	mtDNA D- loop (SS)				mtDNA D-loop (SW)			
	SNP	Amino acid change	Syn/non-syn	Mutation types	SNP	Amino acid change	Syn/non-syn	Mutation types
1	68A>G	Ser23Asn	non-syn	Transition	2C>T	Ala1Val	Non-syn	Transition
2	71A>C	Gln24Pro	Non-syn	Transversion	6T>G	Tyr2STP	Non-syn	Transversion
3	117G>A	Lys39Lys	Syn	Transition	13T>G	Tyr5Asp	Non-syn	Transversion
4	155T>A	Leu52STP	Non-syn	Transversion	14A>G	Tyr5Cys	Non-syn	Transition
5	181G>C	Lys60Asn	Non-syn	Transversion	16C>G	His6Glu	Non-syn	Transversion
6	205T>A	Ser68Arg	Non-syn	Transversion	17A>G	His6Arg	Non-syn	Transition
7	226A>C	Gln75His	Non-syn	Transversion	18C>G	His6Glu	Non-syn	Transversion
8	232G>C	Ser77Ser	Syn	Transversion	22T>A	STP8Lys	Non-syn	Transversion
9	243A>C	Gln81Pro	Non-syn	Transversion	24A>T	STP8Tyr	Non-syn	Transversion
10	254T>C	STP85Gln	Non-syn	Transition	26T>A	Phe9Tyr	Non-syn	Transversion
11	256G>A	STP85Gln	Non-syn	Transition	30G>A	MET10Ile	Non-syn	Transition
12	257T>C	STP86Gln	Non-syn	Transition	34A>T	Asn12Tyr	Non-syn	Transversion
13	295T>C	Gly98Gly	Syn	Transition	35A>G	Asn12Ser	Non-syn	Transversion
14	420T>A	Val140Asp	Non-syn	Transversion	41C>G	Ser14Cys	Non-syn	Transversion
15	486T>C	Val162Ala	Non-syn	Transition	50G>A	STP17Tyr	Non-syn	Transversion
176	176SNPs	176	155/21	108/68	162SNPs	162	148/14	90/72

The myriad of biological data used for genetic diversity analysis, sequence data have proven to be more informative in resolving genetic differences and relatedness among species. Of interest are the sequence data from mitochondrial DNA. According to (Habib *et al*., 2010), mtDNA variation is being adopted as a dependable tool for determining genetic diversity within and among species. This study becomes imperative owing to the observed genetic erosion in tilapia fish orchestrated by the continuous over-exploitation from the wild by local fishermen as well as a low research interest, which has hampered breeding, cultivation and improvement. Our findings showed that the polymorphic sites for mtDNA D-loop (S-S), D-loop (S-W) and Cyt-b (S-S) were 176, 162 and 144, respectively with parsimony information site of 99, 124 and 112. There were also variations in the haplotype and nucleotide diversity. Abdul *et al.* (2015) reported a haplotype diversity of 1.00 in four tilapia species using mtDNA D-loop sequence. Agbebi *et al.* (2016) reported haplotype and nucleotide diversity of 0.232 and 0.00321 in Terubok fish using Cyt-b sequence, which was lower than the present result using Cyt-b sequence. The implication therefore, is that different fish species have varying haplotype and nucleotide diversity using the same region of the mtDNA. The relatively high haplotype and nucleotide diversity observed in this study might suggest high molecular differences within and between the populations (Liu *et al*., 2007).

Single nucleotide polymorphism (SNP) represents the most widespread type of sequence variation in genome, which has emerged as valuable genetic markers for revealing the evolutionary history of populations (Brumfield *et al*., 2003). 176, 162 and 144 SNPs were detected from the three tilapia populations studied, which corresponds to the number of polymorphic sites earlier reported. For all the SNPs detected, non-synonymous substitution was higher than synonymous substitution giving rise to higher positive selection. What it does therefore suggest is that evolutionary distance based on non-synonymous substitutions is expected to be greater than synonymous substitutions. According to (Pennings and Hermission, 2006), there could be the occurrence of selective sweep, which reduces or eliminates the variation among the nucleotides near a mutation in DNA. This might be due to a beneficial alleles having recently reached fixation as a result of strong positive natural selection. We report negative Tajima's D value for mtDNA D-loop tilapia fish samples (-1.080 and -0.83) while mtDNACyt-b region had a positive Tajima's D value of 0.673 suggesting population size expansion and sudden population contraction for D-loop and cyt-b of the tilapia fish, respectively.

Table 3: Mutation analysis of single nucleotide polymorphism (SNPs) in mtDNA D-loop and Cyt-b of tilapia fish from south south, Nigeria

S/N	MtDNA D- loop(SS)				mtDNACyt- b(SS)			
	SNP	Amino acid change	Syn/non-syn	Mutation types	SNP	Amino acid change	Syn/non-syn	Mutation types
1	68A>G	Ser23Asn	non-syn	Transition	4A>G	Lys2Gly	Non-syn	Transition
2	71A>C	Gln24Pro	Non-syn	Transversion	5A>G	Lys2Gly	Non-syn	Transition
3	117G>A	Lys39Lys	Syn	Transition	14T>G	Ile5Arg	Non-syn	Transversion
4	155T>A	Leu52STP	Non-syn	Transversion	24T>G	Ser8Arg	Non-syn	Transversion
5	181G>C	Lys60Asn	Non-syn	Transversion	26T>G	Phe9Cys	Non-syn	Transversion
6	205T>A	Ser68Arg	Non-syn	Transversion	28T>G	Cys10Gly	Non-syn	Transversion
7	226A>C	Gln75His	Non-syn	Transversion	39T>G	Phe13Leu	Non-syn	Transversion
8	232G>C	Ser77Ser	Syn	Transversion	40A>T	Asn14Tyr	Non-syn	Transversion
9	243A>C	Gln81Pro	Non-syn	Transversion	45G>A	Gln15Gln	Syn	Transition
10	254T>C	STP85Gln	Non-syn	Transition	73T>G	Leu25Val	Non-syn	Transversion
11	256G>A	STP85Gln	Non-syn	Transition	135C>A	Arg45Arg	Syn	Transversion
12	257T>C	STP86Gln	Non-syn	Transition	815T>C	Phe272Ser	Non-syn	Transition
13	295T>C	Gly98Gly	Syn	Transition	817C>G	Arg273Gly	Non-syn	Transversion
14	420T>A	Val140Asp	Non-syn	Transversion	818G>T	Arg273Leu	Non-syn	Transversion
15	486T>C	Val162Ala	Non-syn	Transition	821C>G	Ala274Gly	Non-syn	Transversion
176	176SNPs	176	155/21	108/68	144SNPs	144	137/7	91/53

Conclusion

The results put together revealed higher nucleotide and haplotype diversity, more SNPs detected, positive selection and unshared haplotype in mtDNA D-loop when compared with mtDNACyt-b region of tilapia fish, giving rise to more polymorphism.

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