DNA FINGERINTS OF TILAPIA SPECIES IN SHATT AL-AREB RIVER USING RAPD MARKERS

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ABSTRACT

In the last decade, tilapia fish species distributed in the Iraqi inland waters. Three species; Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758), Blue tilapia *Oreochromis aureus* (Steindachner, 1864) and Redbelly tilapia *Coptedon zillii* (Gervais, 1848) inhibiting Shatt Al-Arab River. They belong to family Cichlidae. They are very similar to differentiate among them using biometry. So, genetic markers used for species discrimination. Randomly amplified polymorphic DNA (RAPD) protocol used to examine genetic variation and to generate DNA fingerprints of tilapia fish species in Shatt Al-Arab River. Sixty-two specimens of tilapia fish collected from Shatt Al-Arab River at the governorate of Basrah. Seven universal decamer primers selected *(OPA08, OPA10, OPA13, OPA17, OPA19, OPB08 and OPC02)* to create RAPD DNA fingerprint. RAPD-PCR amplification carried out and electrophoresed with 100 bp ladder. DNA bands scored and molecular weight was calculated using PhotocaptMW software. Analog histogram drew using MS-Excel. The three RAPD DNA profiles apparently were different. DNA bands scored in the three species were 67 bands. The size of DNA bands was ranged from 64 bp to 2344 bp. RAPD fingerprints were efficient to distinguish the three species of tilapia fish. DNA markers of the three species of tilapia fish can use to achieve conservation programs of fish species in the future.

Keywords- DNA Fingerprints, RAPD, Tilapia, Fish, Iraq, Shatt Al-Arab.

مجلة العلوم الزراعية العراقية -2020 :51 (4):1082-1087 فداغ وآخرون

بصمة DNA لأنواع أسماك البلطي في نهر شط العرب بأستخدام المؤشرات الجينية العشوائية.

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المستخلص

في العقد الأخير, أنتشرت أنواع من أسماك البلطي في المياه الداخلية في العراق. وقد سجل منها ثلاث أنواع هي: البلطي النيلي *Oreochromis niloticus* (Linnaeus, 1758) والبلطي الأزرق *Oreochromis aureus* (Steindachner, 1864) والبلطي الأحمر *Coptedon zillii* (Gervais, 1848) وهي تقطن نهر شط العرب. وهي تنتمي الى عائلة Cichlidae . وأن هذه الأنواع الثلاثة متشابهة مظهريا بحيث يصعب التفريق فيما بينها باستخدام الصفات المظهرية. أستخدمت المؤشرات الجينية للتفريق بين الأنواع. أتبعت تقنية التضخيم العشوائي للحامض النووي DNA. فحصت التغيرات الجينية فيما بين الأنواع الثلاثة وتحديد بصمة DNA للأنواع الثلاثة. جمعت 62 عينة من أسماك البلطي من نهر شط العرب في محافظة البصرة. أستخدمت سبع بادئات عشوائية عشرية النيوكليوتيدات *(OPA08, OPA10, OPA13, OPA17, OPA19, OPB08 and OPC02)*. أجريت تجربة التضخيم العشوائي وهجرت نواتج تفاعلPCR كهربائيا بالترافق مع مسطرة جزيئية مقسمة لكل 100 زوج قاعدي. حسبت حزم DNA وقدر الوزن الجزيئي بأستخدام برنامج Photocapt-MW. رسم هستوكرام مماثل للصور بأستخدام نظام الأكسل. أظهرت صور الترحيل الكهربائي DNA العشوائية أختلافا بين الأنواع الثلاث. حسبت 67 حزمة في المعاملات الثلاث وأن حجم الحزم تراوح من 64-2344 زوج قاعدي. وقد أظهر البحث ان طريقة المؤشرات الجينية كفوءة للتميز بين الأنواع الثلاثة من أسماك البلطي. ويمكن أن تستخدم النتائج في برامج حفظ الأنواع في المستقبل.

الكلمات المفتاحية: بصمة DNA, طريقة التضخيم العشوائي, اسماك, البلطي, العراق, شط العرب.

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INTRODUCTION

Inland waters of southern Iraq are a suitable habitat for the native fishes. While exotic fishes were adapted and became members of the aquatic environment component. In the last decade, from an unknown source, tilapia fish species distributed in the Iraqi inland waters. Redbelly tilapia *Coptedon zillii* (Gervais, 1848) recorded in Euphrates River near Al-Musaib town at the governorate of Babel (23). While Mutlak and Al-Faisal (21) recorded Blue tilapia *Oreochromis aureus* (Steindachner, 1864) and Redbelly tilapia *Coptedon zillii* in Shatt Al-Arab River in the governorate of Basrah southern Iraq. Furthermore, Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) recorded in Shatt Al-Arab (4). The three tilapia species (Cichlidae: Perciformes) are very similar in morphological characters so it is complicated to distinguish them by non-taxonomists using morphology. In more recent time electrophoresis of proteins used to differentiate among tilapia species (26). Nevertheless, protein method failed to distinguish among *Oreochromis niloticus* subspecies (24). Recently Genetic markers of mitochondrial DNA are successfully used to differentiate among Nile tilapia *O. niloticus* subspecies using restriction fragment length polymorphism (RFLP) (1). Whereas RFLP needs information about the DNA sequence of a gene and the restriction sites (19). While random amplified polymorphism DNA (RAPD) is, an arbitrary protocol depends on polymerase chain reaction (PCR) technique. In this protocol, undefined segments of the genome were amplified using arbitrary primers (29) and short oligonucleotides often ten (30). RAPD can detect the genetic variation among species without previous knowledge of genome sequence (17). While the number and values of amplified bands depend on the sites of the genome that short primers anneal within. The number of bands separated by gel electrophoresis creates a DNA fingerprint of that species, population or individual. RAPD markers used extensively to create DNA fingerprints of fish species. Therefore, Tilapia genera and species in Egypt were distinguished by RAPD markers (2). In the other side, Baradacki and Skibinski (6) identified tilapia species and subspecies. Dinesh *et al*. (11) analyze tilapia species fingerprints. Furthermore, RAPD markers used to differentiate among Spanish *Barbus* species (8), some Iberian cyprinid species (9, 10), European sea bass populations (7), striped red mullet populations (20). Genetic similarity and diversity of cultured catfish (*Silurus asotus*) populations were analyzed (31) and *Etroplus maculatus* populations (18).Locally the RAPD markers were efficient to differentiate among Iraqi cyprinid fish species (15). Furthermore, it could separate the genus *Barbus* species from another related genus (13). On the population level, *Luciobarbus xanthopterus* from four freshwater environments differentiated genetically using couple RAPD markers (14). In the other side, carangid fish species from Iraqi marine waters also investigated by RAPD markers (3). The present study aimed to create DNA fingerprints of three tilapia species of Shatt Al-Arab River, differentiate among them, investigate genetic-relationship with Euphrates tilapia population and establish to molecular identities for them.

MATERIALS AND METHODS

Sixty-two samples of Tilapia fish *Oreochromis niloticus, Oreochromis aureus* and *Coptedon zillii* collected of the Northern of Shatt Al-Arab River. Fishes put in a cool box filled with ice. They transferred to the laboratory and preserved under -20° C until use. Primary, they classified to the genus and species levels depend on morphological characters (12). For lab work, caudal fins cut and preserved in 95% ethanol alcohol. Genomic DNA extracted from caudal fin pieces (3-5 mg) using DNA Extraction Kit (Genaid) the manufacturer manual was followed. The products of Extraction were well preserved under -20˚C temperature. Extraction products tested by electrophoresis on 1% agarose gel and voltage 70V using ethidium bromide dye and bromophenol blue as loading dye. The genomic DNA products with seven RAPD universal primers (Table 1) used in PCR. In addition, the PCR program used on thermocycler as in table (2).

Table 1. RAPD Primers used in the PCR reaction of Tilapia species

No.	name	Seq.	GC%
P1	OPAO8	GTGACGTAGG	60
P2	OPA10	GTGATCGCAG	60
P3	OPA13	CAGCACCCAC	70
P4	OPA17	GACCGCTTGT	60
P5	OPA19	CAAACGTCGG	60
P6	OPB08	GTCCACACGG	70
P7	OPC02	GTGAGGCGTC	70

Table 2. Program of thermocycler PCR used in RAPD reactions

Stage	Function	Step	Temp.℃	Time (min.)	Cycles
1	Initial denaturation	1	95	5	1
2	Denaturation	1	95	1	35
	Annealing	2	36	1	
	Elongation	3	72	1	
3	Final elongation	1	72	6	1

The PCR products were examined (with 100 bp ladder) on agarose 1.5% gel electrophoresis on voltage 70V for 40-50 minutes and checked in gel documentation UV light plate and photographed by Galaxy mobile Camera. The molecular weight of DNA bands calculated by PhotoCapt.MW software. Furthermore, Microsoft Office Excel used to draw histogram analog to the RAPD profile using the molecular weight of DNA bands results. While the interspecific relationship among tilapia species analyzed by UPGMA online (28).

RESULTS AND DISCUSSION

Results of this study showed three different RAPD DNA profiles of tilapia species *Oreochromis niloticus, Oreochromis aureus* and *Coptedon zillii* inhabiting the Shatt Al-Arab River. They well-differentiated due to the DNA bands distribution that amplified with each primer. While the histogram analog showed the band volume more accurately. The number of bands calculated in the three figures (1, 3, and 5) of PCR products of the three reactions electrophoresed on agarose gel was 67 bands. The band size ranged from 64 to 2344 bp. The results showed the three different haplotypes indicate three distinct fish species *Oreochromis niloticus, Oreochromis aureus* and *Coptedon zillii* as in fig 1, 3 and 5. While the histogram analog reveals the difference in band size (fig. 2, 4 and 6). The number of bands generated per primer in three species varied between 7 and 12 bands. The dominant scorable bands were calculated but the faint bands excluded. Primer P5 was the most producible primer among the seven RAPD primers used in this study since the number of produced bands was 12. While the minimum number (seven bands) produced by P4.

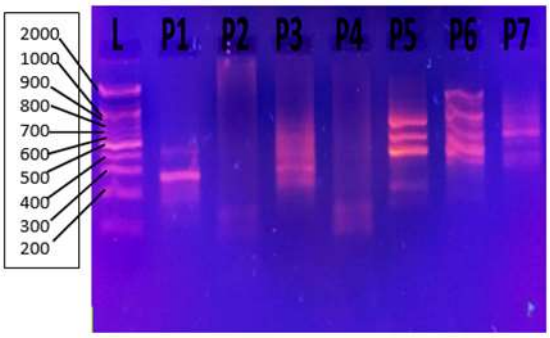

Fig 1. DNA fingerprint of *Oreochromis niloticus* electrophoresed on %2 agarose gel, Voltage 70V using RAPD primers. L: Ladder, P: primer

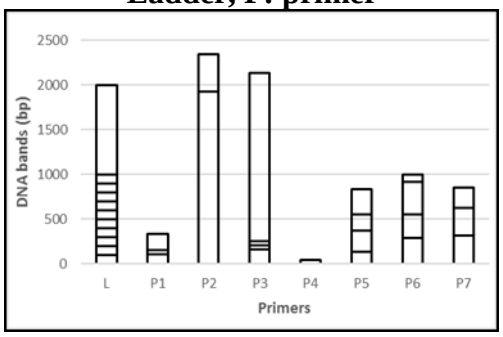

Fig 2. Profile analog of DNA fingerprint of *Oreochromis niloticus* electrophoresed on %2 agarose gel, Voltage 70V using RAPD primers. L: Ladder (bp), P: primer

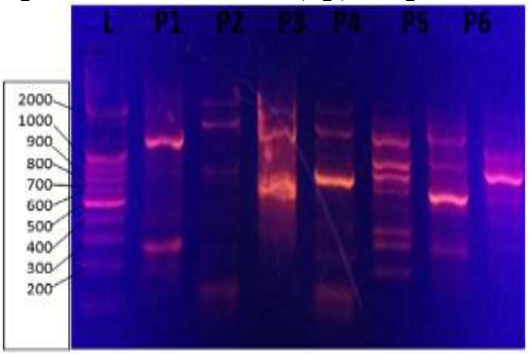

Fig 3. DNA fingerprint of *Oreochromis aureus* electrophoresed on %2 agarose gel, Voltage 70V using RAPD primers. L: Ladder, P: primer

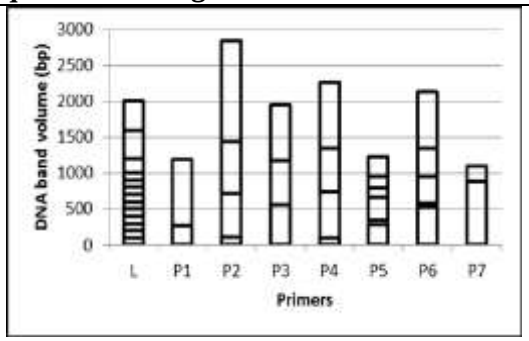

Fig 4, Profile analog of DNA fingerprint of *Oreochromis aureus* electrophoresed on %2 agarose gel, Voltage 70V using RAPD primers. L: Ladder (bp), P: primer

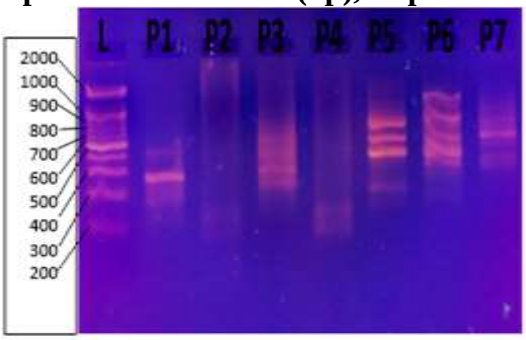

Fig 5. DNA fingerprint of *Coptodon zillii* electrophoresed on %2 agarose gel, Voltage 70V using RAPD primers. L: Ladder, P: primer

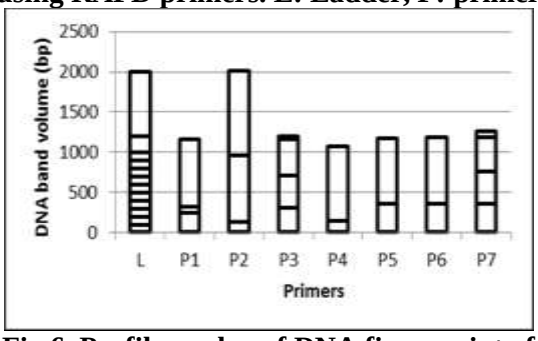

Fig 6. Profile analog of DNA fingerprint of *Coptodon zillii* electrophoresed on %2 agarose gel, Voltage 70V using RAPD primers. L: Ladder (bp), P: primer

The molecular profiles obtained from the 2% agarose gel electrophoresis of PCR product documentation for the three tilapia species appeared that all of the studied species responded to the seven RAPD primers used in PCR processes, annealed and amplified dominant and recessive bands in their PCR products (fig.1, 3 and 5). The Three species O. *niloticus*, O. *aureus*, and C. *zillii* varied in the number of bands created by the seven decamer primers since they ranged from 20 bands in T. z*illii* to 26 bands in O. *aureus*. Whereas, DNA fingerprints of tilapia fish species using randomly amplified polymorphic DNA (RAPD) protocol carried out for the first time in Iraq to examine genetic variation among them. The results of the three groups of samples analysis differentiate the three species completely. While there is genetic relatedness (fig. 7) between these tilapia populations and the sister populations in Euphrates in governorate of Al-Muthanna (5). In the technical aspect, the (RAPD) protocol was reliable, simple to set up, fast and large areas of genomic DNA screened as the study proved. Therefore, the study agrees with earlier studies used the same protocol (17). As well as, needs a minute amount of DNA, no prior information about DNA sequence required as that required in the study compared to other techniques. These advantages make it more preferable than other techniques. The study agrees with local study accomplished to create DNA fingerprints of some cyprinid fish species in Iraq (15). The RAPD-PCR amplification with a single decamer primer to produce DNA fingerprint was affecting essentially with primers, DNA template and reaction conditions. On the other hand, this method needs accurate laboratory work, and multiple decamer primers should utilize to generate a spectrum of molecular markers to establish fingerprints. So that these advantages in comparison to other DNA fingerprinting method, such as restriction fragment length polymorphism (RFLP) (16) make RAPD preferable technique. Finally, a baseline of genetic studies on tilapia fish in Shatt Al-Arab River has been established to continue to other progressive studies with more current techniques like DNA barcoding. DNA markers of the three species of tilapia fish can use to achieve conservation programs of fish species in the future.

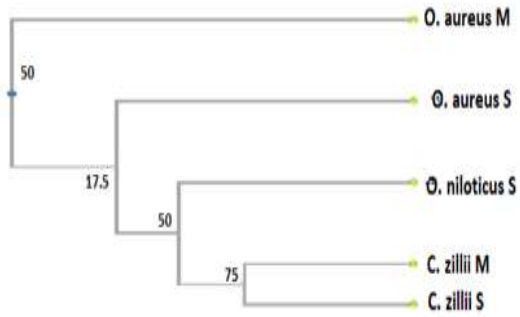

Figure 7. UPGMA dendrogram of the genetic relationship of three tilapia species of Shatt Al-Arab River in the governorate of Basrah (S) and two tilapia species of Euphrates in the governorate of Al-Muthanna (M).

The scenery of the river including water quality, temperature, bottom properties affect the living organisms (27). Therefore, estuarine Shatt Al-Arab River characteristics differ than other aquatic ecosystems. That would affect the phenotypes of tilapia populations. While the genetic markers method succeeded to differentiate genotypes of species and populations. Actually, using the RAPD genetic markers was beneficial in creating specific genetic fingerprint to distinguish among species of the tilapia. This results also reported by Shair *et al*. (25)who studied three tilapia cultured species in Saudi Arabia using single RAPD primer for differentiation among the three species and creating genetic fingerprints to each one which can be considered specific for them. While the variation in bands number and volume indicate to the genetic distance among the studied fish species and the presence of the same bands in more related species explain the evolutionary relationships among fish species (22).

CONCLUSION

DNA fingerprints among species and relative variation among populations due to the different environments. In the second hand, using Random Amplified Polymorphic DNA (RAPD) to create DNA fingerprint gave considerable results. In the same time, the RAPD method was easy, efficient and inexpensive in comparison with other methods. RAPD markers distinguished the genetic variation among tilapia populations, according to geographic isolation. Therefore, studying the genetic relationships among Iraqi fishes on the species and population levels using RAPD markers is useful in order to investigate the genetic diversity among the fish species in Iraqi waters.

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