# MUSCLE PROTEIN AND LIVER ESTERASES BANDING PATTERNS AS BIOCHEMICAL MARKERS TO DETERMINE GENETIC DIVERSITY IN EGYPTIAN POPULATIONS OF *Tilapia* SPECIES

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*Tilapia* is the most species for fresh water aquaculture and its original habitat is Africa and the southwestern Middle East, even spread in worldwide. It is known as one of the main sources of animal protein in the future. Usually live in fresh water, also can live in different salt content of the water. It has a strong adaptability, in the waters of an area of narrow also can breed Badawy (1998), even in the rice fields to grow and have strong adaptability to less dissolved oxygen in the water. The vast majority of *tilapia* is omnivorous, eating water plants and debris (El-Tawil, 1984; Ladewig and Shwantes, 1984; Bezrukov 1987).

Many studies were conducted using protein banding patterns to determine the biodiversity between and among species of different fish genera. El-Ghobashy *et al*. (2005) studied the similarity between carp fish populations based on soluble muscle protein whereas Rashed *et al*. (2007) determined the polymorphism among some *Hemichrois bimaculatus* fish populations using muscle protein polymorphism. Moreover; the biochemical genetic marker is useful for breeding programs for *Clarias gariepinus* (Saad *et al*., 2009). Regarding *Tilapia sp*, Eshak *et al*. (2010) assessed three generations of red *tilapia* from Maryut Lake during 2008/2009 season for salinity tolerance. The results showed that protein banding pattern between individuals or between generations showed the highest similarity on different test organs. The polymorphism from protein electrophoretic pattern was 65.5%.

The banding patterns of isozymes were adopted for many scientific purposes. Debnath (1978), Sahib and Rao (1980) and Shengming *et al*. (1988) reported that esterases are used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture, maintaining normal physiology, metabolism, detoxifying various drugs and environmental toxicants in living systems.

Kijima and Fujio (1990), Jean *et al*. (1995), Barua *et al.* (2004), El-Ghobashy *et al*. (2005), Silva *et al*. (2008), Mugiyanto *et al*. (2013), Mustafa *et al*. (2013) and Rashid and Rahman (2013) reported that the isozymes, tissues, buffer system are suitable for analyzing and studying the interspecific phylogenetic relationships of different species and subfamilies of different fish genera. Meanwhile; Rognon *et al*. (1996), Saad *et al*. (2009) and Bakhoum *et al*. (2009), studied

*Egypt. J. Genet. Cytol., 45:187-203, July, 2016*
Web Site (*www.esg.net.eg*)

polymorphism in natural and cultured populations in some *Tilapia* species and be a useful tool in studying fish genetic structure. Esterase isozymes were expressed in liver and stomach and separated to five bands in *Oreochromis niloticus* and isozymes were used in studying of the population genetic structure, identification of fish species and estimation of genetic variation between populations of fish species. phylogenetic relationship between the intermediate morphological forms and their putative parents *O. niloticus* and *O. aurea*. The number of the genes controlled esterase isozymes was five loci in both organs of *O. niloticus* and hybrid types while *O. aurea* gave four loci in liver and five loci in kidney (Shahjahan *et al*., 2008; Silva *et al*., 2008; Bakhoum *et al*., 2009).

This study aimed to estimate the genetic diversity, population differentiation and population structure among and within populations from different locations of three *Tilapia* species based on flesh protein and liver esterases banding patterns.

## MATERIALS AND METHODS

### *1-Fish collection*

Three *Tilapia* species, *i.e., O. niloticus*, *O. aurea* and *T. zilli* collected from three different locations were used in this study. Two of these locations belonging to Kafr El-Sheikh Governorate (3748.12 $km^2$), *i.e.*, Ryad (locates at Middle of Kafr El-Sheikh Governorate) and Motobs which located at North-West of Kafr El-Sheikh Governorate and adjacent to Mediterranean Sea, Al-Borolos lake and Nile River. The third one was from Bahr El-Baqar from El-Sharqia Governorate. Ten individuals from each population were used except Bahr El-Baqar population of *O. aurea* where five individuals were used according to sampling limitation.

### *2-Biochemical studies*

This study was done according to El-Fadly *et al.* (1990) with some modification. Two hundred milligram of fish muscle for protein detection was homogenized in one ml of NaCl 0.85% solution. One hundred mg of fish liver for isozyme detection was homogenized in one ml of sucrose 20%.

The extracts were incubated at -20°C for half hour and were then centrifuged under cooling (4°C) at 12000 rpm for 25 min using Hettich EBAR 12 centrifuge. The clear supernatant were transferred to new eppendorf tubes and stored at -20°C until use. From each sample, 400 µl supernatant was transferred to 100 µl sample buffer (5x) and followed by heating at 95°C for 10 min for muscle protein only.

SDS-PAGE preparation, staining and distaining were prepared according to laemmli (1970).

Isozyme electrophoresis solutions, native PAGE preparation and conditions were carried out according to Stegmann *et al*. (1985). Esterases (EST) Isozyme developing reagents and conditions were

carried out according to Scandalios (1964).

### *3- Data scoring, analyzing and similarity estimation*

All of the obtained electrophoretic bands either from protein or esterases isozymes were scored as (-) absent, (a) very faint, (b) faint, (c) dark or (d) very dark for their intensity. Afterwards the protein profiles and isozyme zemograms were documented photographically.

Electrophoretic banding patterns of either protein or esterases isozymes profiles were compared with each other, where (1) means presence and (0) means absence of bands regardless their intensity. Data was then analyzed using the PAST, Ver. 1.90 (Hammer *et al*., 2001). The data were used to estimate genetic similarity. Pairwise comparisons between individuals were made to calculate the Jaccard's coefficient using PAST program. Clusters analysis was performed to produce a dendrograms using un-weighted pair-group method with arithmetical average (UPGMA).

## RESULTS AND DISCUSSION

### *I-A Muscle protein electrophorasis*

Figure (1) and Table (1) presented a photograph of electrophoretic protein banding pattern of *O. niloticus* individuals and classification of it according to its density and molecular weight. Data in Table (1) showed that a total of 20 different bands were detected from the three populations *i.e*., Ryad, Bahr El-Baqar and Motobs. Meanwhile not all of the 20 bands appeared in all of them.

Regarding detecting the biodiversity among the three *O. niloticus* populations which were collected from three different locations, data in Fig. (1) and Table (1) clearly showed that 13 bands out of the 20 ones (bands no. 1, 2, 4, 5, 7, 9, 10, 11, 12, 13, 15, 16 and 17) were common bands among the three populations. Moreover, band 8 and 18 were common bands between Ryad and Bahr El-Baqar. Finally band 19 and 20 exhibited special relation between Bahr El-Baqar and Motobs. Band 14 is considered as unique band in Bahr El-Baqar population.

Figure (2) and Table (2) showed a photograph of electrophoretic protein banding pattern of *T. zilli* individuals and classification of them according to their density and molecular weight. Data in Table (2) exhibited that a total of 21 different bands were detected from the three populations *i.e.*, Ryad, Bahr El-Baqar and Motobs. In the same time, not all of the 21 bands appeared in the three populations. Motobs population missed band no. 2 and Ryad population missed bands no. 4, 7, 9 and 11. Bahr El-Baqar populations lost bands no. 3, 6, 10, 14, 15, 16 and 21.

Regarding detecting the biodiversity between and among the three *T. zilli* populations which were collected from three different locations. Data in Fig. (2) and Table (2) clearly showed that 8 bands out of the 21 (bands no. 1, 5, 8, 12, 13, 18, 19 and 20) were common bands among

the three populations. Moreover, bands 2 and 17 were common bands between Ryad and Bahr El-Baqar whereas band 14 exhibited special relation between Ryad and Motobs. Bands no. 4, 7, 9 and 11 appeared in Bahr El-Baqar and Motobs. Exacting appearance of band 3, 6, 10, 15, 16 and 21 in Ryad and completely absence in Bahr El-Baqar and Motobs locations.

Data in Fig. (3) and Table (3) showed a photograph of electrophoretic protein banding pattern of individuals from *O. aurea* and the classification of them according to their density and molecular weight. Data in Table (3) showed that 10 out of the 24 bands (bands no 2, 3, 9, 13, 14, 15, 16, 19, 20 and 21) were common bands in all of the three populations. In Ryad population, fifteen bands were detected with variable intensity from very faint to very dark. Meanwhile, bands no. 1, 16 and 24 were absent in some individuals and found in some others. Regarding to Bahr El-Baqar population, 16 bands out of the 24 ones were detected in all of individuals under study with exception of individual 4 since the first band (200 KDa) was absent in it. Concerning Motobs population, 18 bands out of the 24 were detected with different intensity except band no. 1 which was absent in individual 4.

Regarding to detecting the biodiversity among the three *O. aurea* populations which were collected from three different locations. Moreover bands no. 4, 10 and 12 were common between Ryad and Bahr El-Baqar populations. Three bands no. 1, 7 and 18 appeared in Bahr El-Baqar and Motobs populations. Band no. 22 appeared as a common band between Ryad and Motobs populations. Bands no. 5, 6, 11 and 17 presented as specific bands in Motobs population whereas bands no 8, 23 and 24 were specific for Ryad population only.

Similar results were obtained by El-Ghobashy *et al*. (2005), Rashed *et al*. (2007) and Saad *et al.* (2009) since they found that some populations have a unique profile by using Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) which exhibited a good agreement with the obtained results from this study. They reported that biochemical genetic marker is useful for detecting genetic diversity which could be used in fish breeding programs.

***I- B. Liver esterases isozyme***

Detection of non-specific esterases isozymes (α & β esterases) from liver extracts was studied in order to use it as differential biochemical marker within and among the three different populations of *Tilapia species* under study.

Data in Fig. (4) and Table (4) presented the electrophoretic photograph and density variation of esterases isozymes from *O. niloticus* individuals.

Maximum of four isozymes bands were detected as very faint, faint, dark, very dark or absent. Ryad and Motobs individuals showed four isozymes. Meanwhile, Bahr El-Baqar exhibited only three

ones. The majority of individuals within or among the three location varied in esterases isozymes activities. This activity variation may be due to variation among the living individuals according to many different reasons. In spite of esterase isozymes 1, 2 and 3 which were common ones in the three populations, the fourth one was specialized for Ryad and Motobs populations.

Figure (5) and Table (5) presented the electrophoretic photograph and density variation of esterases isozyme from *T. zilli* individuals. Four isozyme bands were also detected and presented as very faint, faint, dark, very dark or absent. Ryad and Bahr El-Baqar individuals showed three isozymes. Meanwhile, Motobs exhibited reactive and showed four isozymes. The majority of individuals within or among the three location varied in esterases isozymes activities.

The electrophoretic photograph and density variation of esterases isozyme from *O. aurea* individuals are presented in Fig. (6) and Table (6). Maximum two isozyme bands were detected in all the three populations. Band 1 was dark in Ryad individuals and ranged from very faint, faint and dark in Bahr El-Baqar and Motobs. Moreover, band two was very dark in the three populations except sample no 2 in the population of Bahr El-Baqar.

Isozymes were used in studying the population genetic structure identification of fish species and to estimate the genetic variation among populations of fish species as reported by Barua *et al.* (2004), Shahjahan *et al*. (2008), Silva *et al*. (2008) and Bakhoum *et al*. (2009). Number of genes those control esterases isozyme were five loci in *O. niloticus* and hybrid types. This difference between the obtained results from this study and those published previously, may be due to the difference between the genetic backgrounds of the applied species in the different studies. On the other hand, *O. aurea* gave four isozymes in liver and five in kidney as mentioned by Bakhoum *et al*. (2009). Saxena and Soranganba (2012) and Rashid and Rahman (2013) found that enzyme activity synchronized with the feeding metabolic, habitat and environmental factors.

***II - Phylogenetic studies***

According to the obtained results of protein and esterases banding patterns for populations of *Tilapia* species from different locations, the scored bands (1 for presence and 0 for absence) were subjected to pair wise comparisons between individuals (complete data of the similarity matrices not shown but its percentages located above the Figures) to calculate the Jaccard's coefficient. Cluster analysis was performed to produce the phylogeny tree for the three populations.

***II- A. Phylogenetic studies based on protein banding patterns***

Figure (7) represented the phylogenetic relationship within and between the studied populations of the three locations concerning *O. niloticus*. The

dendrogram showed that Ryad and Bahr El-Baqar populations were closely related to each other and share a common ancestor with similarity of about 79% whereas the relationship between both Ryad and Bahr El-Baqar populations with Motobs population exhibited similarity reached to 76%. It seems to be that population of Bahr El-Baqar was highly differentiated than other populations.

Furthermore, data in Fig. (8) represent the phylogenetic relationship within the studied populations of the three locations concerning *T. zilli*. The dendrogram showed that Motobs and Bahr El-Baqar populations were closely related to each other with similarity up to 82%. Meanwhile, individual no. 6 in Motobs popula was closely similar to Bahr El-Baqar individuals (1, 2 and 7) with similarity 90%. Meanwhile, both of Motobs and Bahr El-Baqar populations exhibited similarity of about 43% with Ryad population.

The phylogenetic relationship of the three locations concerning *O. aurea* exists in Fig. (9). The dendrogram showed that Bahr El-Baqar and Ryad populations were closely related to each other with similarity up to 68%. Eight individuals of Motobs population exhibited 100% similarity whereas the similarity with the other two individuals in the same population was 94%.

### *II- B. Phylogenetic studies based on esterases banding patterns*

The isozyme esterase has been used to generate the phylogenetic tree within populations of *O. niloticus* from liver as shown in Fig. (10). Motobs individuals have been separated into the three clusters. Most of Ryad and Motobs individuals separated into two clades and were closely related to each others with similarity reached 74%. On the other hand, Bahr El-Baqar individuals plus two individuals from both Motobs and Ryad were separated in one clade with similarity up to about 66% with the other two cades.

Data in Fig. (11) represent phylogenetic relationship from esterases from liver within populations of *O. zilli.* Individuals of Bahr El-Baqar and Ryad were combined in two clades whereas individuals from Motobs were separated in one clad. Six individuals from Bahr El-Baqar and five from Ryad were closely related to Motobs individuals with similarity reached 71% whereas, the similarity of those two clades with the third clad (containing four individuals from Bahr El-Baqar and five from Ryad) were 61%.

## SUMMARY

Genetic diversity across *Tilapia* species is important key for development aquaculture strains, protection of endangered populations and biogeographical inferences. Total soluble protein and esterases isozymes were extracted from flesh (muscles) and liver of all individuals from the three populations (Ryad, Bahr El-Baqar and Motobs) from each species under study to estimate the genetic diversity. With comparing the obtained bands from the three *Oreochromis niloticus*, it was found that 13 bands out of the 20

were common bands among the three populations. The three *T. zilli* populations exhibited 8 bands out of the 21 were common bands among those three populations. The three *O. aurea* populations exhibited 10 bands were common between three populations, while the rest of bands appeared in some of population and disappeared in the other. Ryad and Motobs individuals of *O. niloticus* showed four isozymes. Meanwhile, Bahr El-Baqar exhibited only three. Ryad and Bahr El-Baqar individuals of *T. zilli* showed three isozymes. Meanwhile, Motobs exhibited four ones. Maximum two isozymes were detected in population of *O. aurea*. Band 1 was dark in Ryad individuals and ranged from very faint, faint and dark in Bahr El-Baqar and Motobs. The phylogenetic relationship within the studied populations of the three locations concerning *O. niloticus*, *T. zilli* and *O. aurea* was conducted and different variations were detected. Population from Ryad was highly differentiated than other populations.

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Table (1): Electrophoretic banding pattern showing the intensity variation of total protein within and among populations of *O. niloticus* from different locations (Ryad, Bahr El-Baqar and Motobs).

| *O. niloticus* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Band No. | M KDa | Ryad | | | | | | | | | | Bahr El-Baqar | | | | | | | | | | Motobs | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | 150 | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 2 | 120 | a | a | a | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 3 | 100 | a | - | - | - | a | a | - | a | - | a | - | - | - | a | - | - | - | - | a | - | a | a | - | - | a | a | - | - | - | a |
| 4 | | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c |
| 5 | 85 | a | a | a | a | a | a | a | a | a | a | - | a | a | a | a | a | - | - | - | a | a | a | a | a | a | a | a | a | a | a |
| 6 | | a | - | - | a | a | a | - | a | a | a | - | - | - | a | a | a | - | - | a | - | a | - | - | a | a | - | a | a | a | a |
| 7 | 60 | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c |
| 8 | | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | - | - | - | - | - | - | - | - | - | - |
| 9 | 50 | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c |
| 10 | 40 | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |
| 11 | 30 | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |
| 12 | | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c |
| 13 | | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 14 | | - | - | - | - | - | - | - | - | - | - | a | b | b | b | b | b | b | b | b | a | - | - | - | - | - | - | - | - | - | - |
| 15 | 25 | a | a | a | a | a | a | a | a | a | a | - | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a |
| 16 | 20 | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 17 | 15 | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 18 | | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | - | - | - | - | - | - | - | - | - | - |
| 19 | 10 | a | - | - | a | - | a | a | - | - | - | b | b | b | a | b | b | a | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 20 | | - | - | - | - | - | - | - | - | - | - | - | b | a | b | a | b | b | a | b | a | b | b | b | a | a | a | b | a | a | a |

(-) absent (a) very faint (b) faint (c) dark (d) very dark

Table (2): Electrophoretic banding pattern showing the intensity variation of total protein within and among populations of *T. zilli* from different locations (Ryad, Bahr El-Baqar and Motobs).

| *T. zilli* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Band No. | M KDa | Ryad | | | | | | | | | | Bahr El-Baqr | | | | | | | | | | Motobs | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | 200 | a | a | a | a | a | a | - | a | a | a | - | - | a | a | a | a | - | a | a | a | a | a | a | a | a | - | a | a | a | a |
| 2 | 150 | b | b | b | b | b | b | b | b | b | b | a | a | - | a | a | a | a | a | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 120 | b | b | b | b | b | b | b | b | b | b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 100 | - | - | - | - | - | - | - | - | - | - | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c |
| 5 | 85 | c | c | c | c | c | c | c | c | c | c | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a |
| 6 | | - | - | a | a | a | a | a | a | a | a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 7 | | - | - | - | - | - | - | - | - | - | - | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 8 | 60 | b | b | b | b | b | b | b | b | b | b | a | b | a | b | a | b | a | b | b | a | b | b | b | b | b | a | b | b | b | b |
| 9 | 50 | - | - | - | - | - | - | - | - | - | - | b | b | b | b | b | b | b | b | b | b | c | c | c | c | c | c | c | c | c | c |
| 10 | | c | c | c | c | c | c | c | c | c | c | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 11 | 40 | - | - | - | - | - | - | - | - | - | - | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |
| 12 | 30 | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |
| 13 | | d | d | d | d | d | d | d | d | d | d | b | b | b | b | b | b | b | b | b | b | c | c | c | c | c | c | c | c | c | c |
| 14 | | c | c | c | c | c | c | c | c | c | c | - | - | - | - | - | - | - | - | - | - | - | - | a | a | a | - | a | a | a | - |
| 15 | | a | b | b | b | b | a | b | b | b | b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | | - | a | a | - | b | b | a | - | b | b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 17 | 25 | a | a | a | a | a | a | a | a | a | a | - | - | - | a | a | a | a | a | a | a | - | - | - | - | - | - | - | - | - | - |
| 18 | 20 | b | b | b | b | b | b | b | b | b | b | a | b | a | a | a | b | a | b | b | a | b | b | b | b | b | b | b | b | b | b |
| 19 | 15 | b | b | b | b | b | b | b | b | b | b | a | b | a | a | a | b | a | b | b | a | a | a | a | a | a | a | a | a | a | a |
| 20 | | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 21 | 10 | - | b | b | a | b | b | b | a | b | a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

(-) absent (a) very faint (b) faint (c) dark (d) very dark

Table (3): Electrophoretic banding pattern showing the intensity variation of total protein within and among populations of *O. aurea* from different locations (Ryad, Bahr El-Baqar and Motobs).

| *O. aurea* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Band No. | M KDa | Ryad | | | | | | | | | | Bahr El-Baqr | | | | | Motobs | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | 200 | - | - | - | - | - | - | - | - | - | a | a | a | a | - | a | a | a | a | - | a | a | a | a | a | a |
| 2 | 150 | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 3 | 120 | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a |
| 4 | 100 | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | - | - | - | - | - | - | - | - | - | - |
| 5 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | c | c | c | c | c | c | c | c | c | c |
| 6 | 85 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | a | a | a | a | a | a | a | a | a | a |
| 7 | | - | - | - | - | - | - | - | - | - | - | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a |
| 8 | 70 | a | a | a | a | a | a | a | a | a | a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | 60 | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 10 | 50 | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | - | - | - | - | - | - | - | - | - | - |
| 11 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | c | c | c | c | c | c | c | c | c | c |
| 12 | 40 | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | - | - | - | - | - | - | - | - | - | - |
| 13 | 30 | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |
| 14 | | c | c | c | c | c | c | c | c | c | c | c | c | c | c | c | d | d | d | d | d | d | d | d | d | d |
| 15 | | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | c | c | c | c | c | c | c | c | c | c |
| 16 | | a | a | a | a | a | a | - | a | a | b | b | b | b | b | b | b | b | b | b | b | b | b | b | B | B |
| 17 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | a | a | a | a | a | a | a | a | a | a |
| 18 | 25 | - | - | - | - | - | - | - | - | - | - | a | a | a | a | a | a | a | a | a | a | a | a | a | a | a |
| 19 | 20 | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 20 | 15 | a | a | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b | b |
| 21 | | a | a | a | a | a | a | a | a | a | a | b | b | b | b | b | a | a | a | a | a | a | a | a | a | a |
| 22 | | a | a | a | a | a | a | a | a | a | a | - | - | - | - | - | a | a | a | a | a | a | a | a | a | a |
| 23 | 10 | a | a | a | a | a | a | a | a | a | a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 24 | | - | - | - | a | a | - | - | a | - | a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

(-) absent (a) very faint (b) faint (c) dark (d) very dark

Table (4): Electrophoretic banding pattern showing the intensity variation of esterases isozymes within and among populations of *O. niloticus* from different locations (Ryad, Bahr El-Baqar and Motobs).

| *O. niloticus* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Band No. | Ryad | | | | | | | | | | Bahr El-Baqr | | | | | | | | | | Motobs | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | b | c | c | c | c | a | c | a | b | c | d | c | d | d | d | d | d | d | b | c | b | d | d | d | a | d | d | d | d | d |
| 2 | - | d | d | d | d | - | - | c | c | c | b | c | - | d | - | d | c | c | - | c | d | d | d | b | b | d | d | b | c | d |
| 3 | - | c | b | - | b | c | c | - | b | c | c | b | c | d | d | d | b | b | c | b | a | c | - | d | b | b | c | c | b | b |
| 4 | d | d | d | c | c | b | c | b | - | - | - | - | - | - | - | - | - | - | - | - | - | d | c | d | c | c | - | d | b | d |

(-) absent (a) very faint (b) faint (c) dark (d) very dark

Table (5): Electrophoretic banding pattern showing the intensity variation of esterases isozymes within and among populations of *T. zilli* from different locations (Ryad, Bahr El-Baqar and Motobs).

| *T. zilli* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Band No. | Ryad | | | | | | | | | | Bahr El-Baqr | | | | | | | | | | Motobs | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | c | c | c | c | c | b | c | b | c | c | c | c | c | c | c | b | c | b | c | c | b | b | b | c | c | d | b | b | b | b |
| 2 | a | a | a | - | - | - | - | - | a | a | a | a | a | - | - | - | - | a | a | a | b | a | - | a | a | a | - | b | a | a |
| 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | b | b | c | c | c | b | c | b | b | b |
| 4 | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | b | c | d | d | d |

(-) absent (a) very faint (b) faint (c) dark (d) very dark

Table (6): Electrophoretic banding pattern showing the intensity variation of esterases isozymes within and among populations of *O. aurea* from different locations (Ryad, Bahr El-Baqar and Motobs)

| *O. aurea* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Band No. | Ryad | | | | | | | | | | Bahr El-Baqr | | | | | | | | | | Motobs | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | c | c | c | c | c | c | c | c | c | c | c | c | a | b | b | c | c | c | b | c | c | c | b | c | c | c | c | c | c | c |
| 2 | d | d | d | d | d | d | d | d | d | d | d | c | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |

(-) absent (a) very faint (b) faint (c) dark (d) very dark

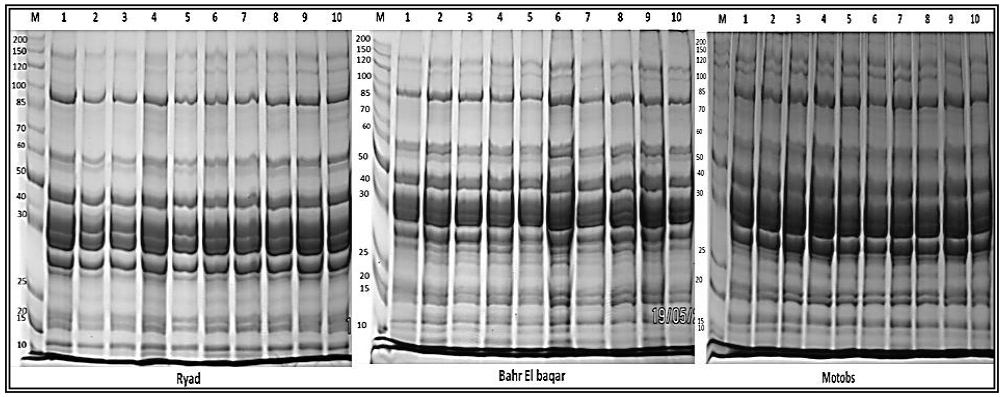


Fig. (1): Protein banding pattern of SDS-PAGE showing diversity between and among population of *O. niloticus* from different locations (Ryad, Bahr El-Baqar and Motobs).

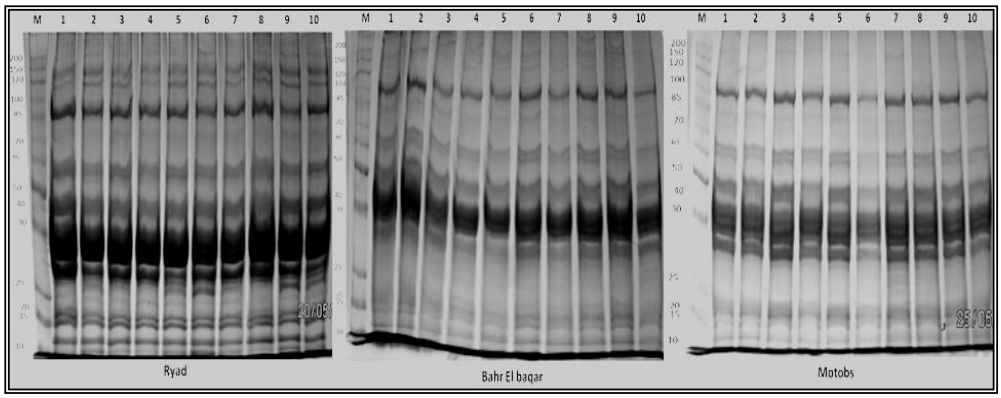


Fig. (2): Protein banding pattern of SDS-PAGE showing diversity between and among population of *T. zilli* from different locations (Ryad, Bahr El-Baqar and Motobs).

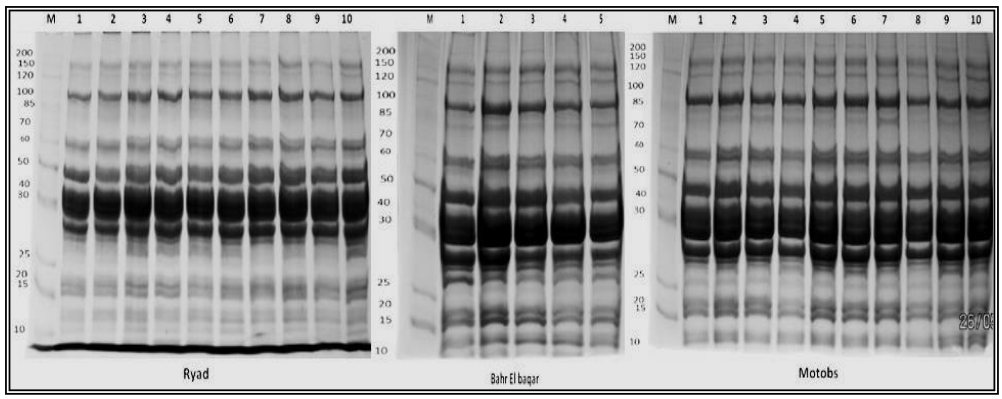


Fig. (3): Protein banding pattern of SDS-PAGE showing diversity between and among population of *O. aurea* from different locations (Ryad, Bahr El-Baqar and Motobs).

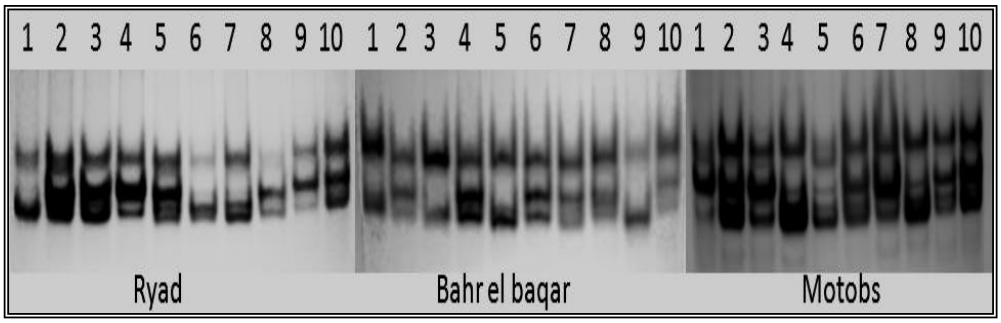


Fig. (4): Comparative esterases isozyme banding pattern within and among populations of *O. niloticus* from different locations (Ryad, Bahr El-Baqar and Motobs).

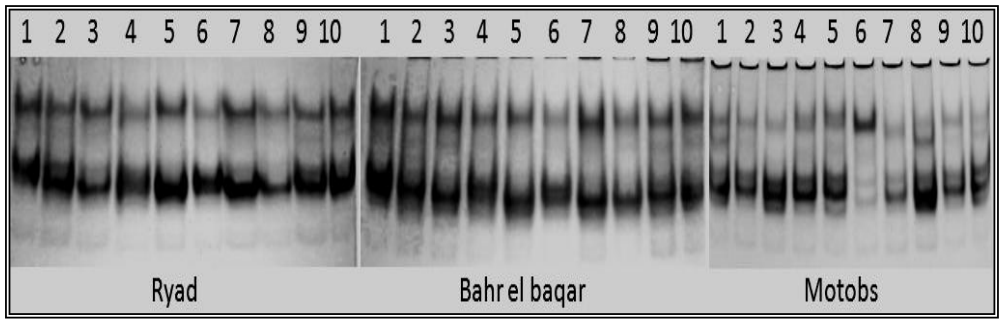


Fig. (5): Comparative esterases isozyme banding pattern within and among populations of *T. zilli* from different locations (Ryad, Bahr El-Baqar and Motobs).

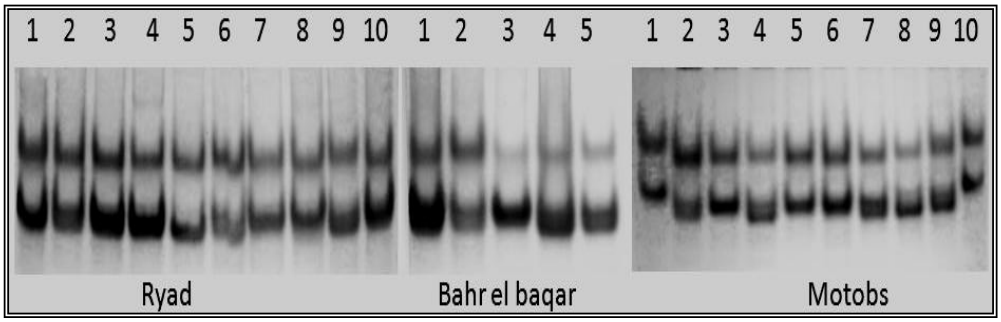


Fig. (6): Comparative esterases isozyme banding pattern within and among populations of *O. aurea* from different locations (Ryad, Bahr El-Baqar and Motobs).

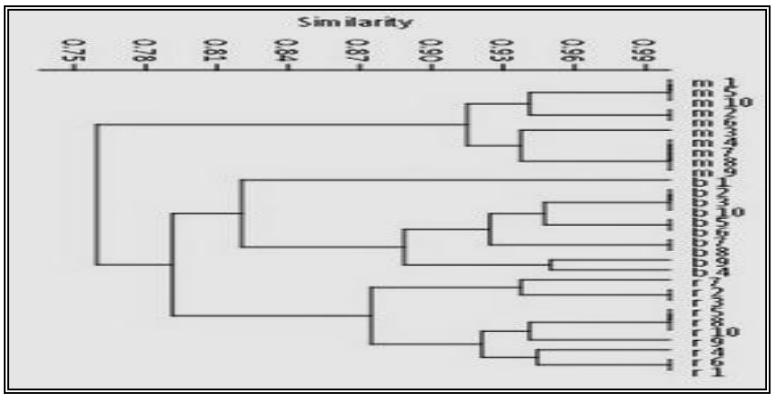


Fig. (7): Dendrogram represent phylogenetic relationship within population of *O. niloticus* from muscle protein data.

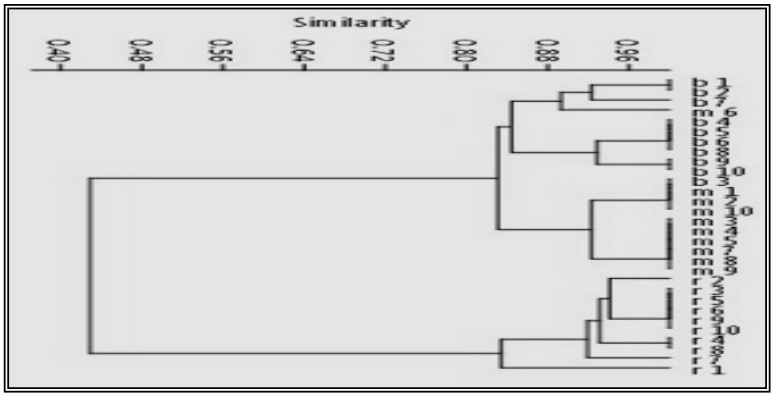


Fig. (8): Dendrogram represent phylogenetic relationship within population of *T. zilli* from muscle protean data.

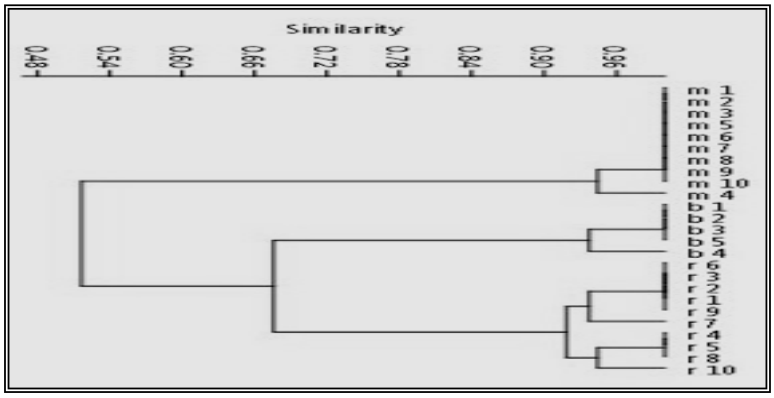


Fig. (9): Dendrogram represent phylogenetic relationship within population of *O. aurea* from muscle protean data.

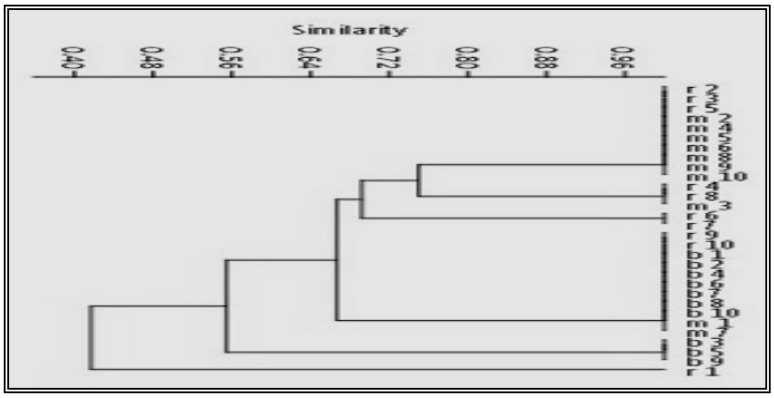


Fig. (10): Dendrogram represent phylogenetic relationship within population of *O. niloticus* from muscle liver esterases data.

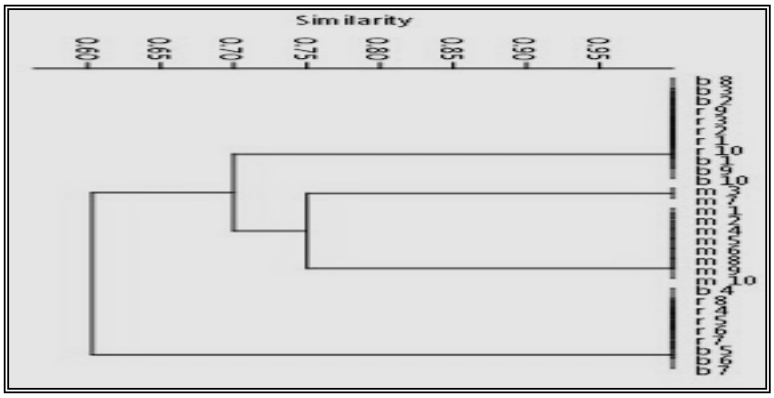


Fig. (11): Dendrogram represent phylogenetic relationship within population of *T. zilli* from muscle liver esterases data.