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IDENTIFICATION OF OREOCHROMIS NILOTICUS IN EGYPT BY THIN LAYER POLYACRYLAMIDE ISOELECTRIC FOCUSING.

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ABSTRACT

The River Nile is considered the main source of **Oreochromis niloticus** in Egypt. In the recent years considerable ecological changes have occurred in the Nile ecosystem and the northern lakes as well, which may affect the species population. Therefore, specimens of **Oreochromis niloticus** were collected from different habitats including Lake Naser, Aswan region, Cairo region, Lake Mariut and Lake Manzallah. Eye lens protein was examined and compared by thin layer isoelectric focusing. It was found that the protein patterns of the fishes from different localities, did not exhibit any significant variation, which may indicate that O. niloticus in the sites under investigation still has the proper protein characters of the pure strain in spite of the ecological conditions.

INTRODUCTION

Oreochromis niloticus is the most economic important fresh water fish in Egypt. Recently, more attention has been paid to it as a partner in monosex fish farm. However, the guarantee of the purity of Oreochromis species is necessary for hybridization purpose (Wu et al., 1983).

The recent conditions of the Nile and the northern lakes after the construction of High Dam and the increase in industrial and agricultural activities, may have an impact on the ecology of the different water masses. For example, the ecology of Lake Naser is to a high degree differnt than that of Lake Manzallah (Ibrahim, 1987 and Elewa, 1987). The differece in ecology had affected the fish. Elewa, (1987) found that O. niloticus in Lake Naser grows to about 20.7 cm in total length by the end of its first year of life, while Hosny (1987) reported that, in Lake Manzallah O. niloticus has a total length of only 9.0 cm. Oberst et al., (1983) stated that the water quality is one of the most important variables in genetic studies.

As the classic taxonmic tools can not help in differentiation between the different populations within the same species, hence, the urgent need for establishing more accurate criteria for species identification must be emphasized (Wu et al., 1983).

Avtalion et al. (1976) studied the serum protein of Tilapia using polyacrylamide gel electrophoresis and their results showed the existence of species-specific markers.

Thin layer isoelectric focusing was found able to give a very good separation and producible patterns of sarcoplasmic protein of different populations within the same species (Ukishima et al., 1984 and Ng et al., 1986). Using this technique, the complex genetic constitution can be clearly correlated with the species-specific protein profiles obtained (Lundstrom, 1981 & 1983; Yamada and Suzuki, 1982; Ukishima et al., 1984 and Kamel, 1987). The present study aims to the eye lens protein profile of Oreochromis niloticus at different ecological sites and to show to what extent this fish is present as a pure strain on applying the isoelectric focusing technique.

MATERIAL AND METHODS

Ten fishes of O. niloticus were collected during April and May 1987 from each of Lake Naser, Aswan region, Cairo region, Lake Mariut and Lake Manzallah, regardless of size or sex (Fig. 1). An additional specimens of a pure strain of O. niloticus and a hyprid of male O. aureus x female O. niloticus were obtained from Fish Research Center, University of Seuz Canal.

For each fish, both eyes were removed and the eye lens was obtained, washed in distilled water for 10 sec. then cut with sharp scalpel to obtain the fluid which was diluted 1:1 with distilled water.

Isoelectric focusing was conducted on an LKB Multiphore II (Model 2117) using Ampholin PAGplate, pH 3.5-9.5 polyacrylamide gel (LKB 1804-101). The constant power was set at 25 w and cooling temprature was set at 10^{0} C. The anode electrode solution was 1M H_3PO_4 and the cathode electrode solution was 1M NaOH. The technique of protein staining was that given in LKB instruction No. 1804.

Sample application:

The eye lens fluids were applied onto the gel using sample application pieces supplied by LKB. The sample concentration was not measured. After sample application, the unit was run for 30 min., after which the application pieces were removed and the isoelectric focusing was continued for 90 min.

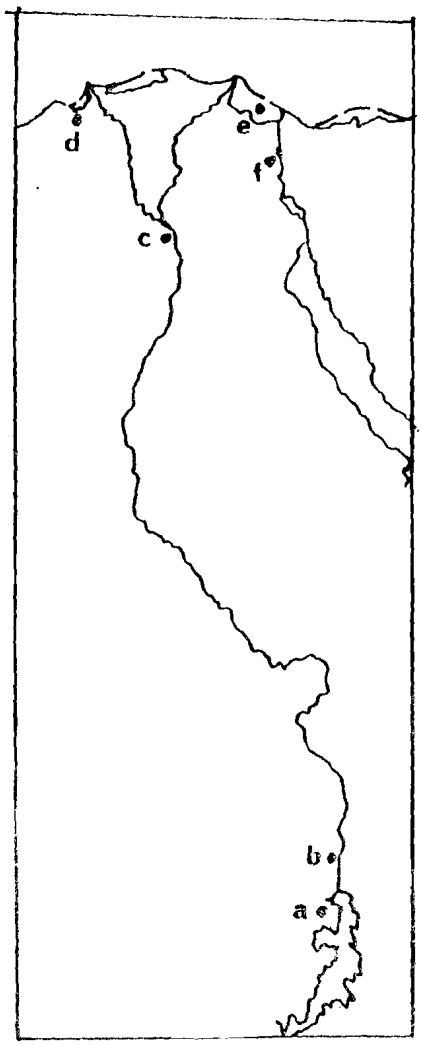

FIG .1.
Different locaations of sample collection.
a, Lake Naser; b, Aswan; c, Cairo; d, lake mariut;
e, Lake Manzallah; f, Ismaelea.

pH measyrement:

After electrofocusing a strip of the gel of 1 cm width was cut for measuring the pH gradient as has been given by Ng et al., (1986).

The pI (isoelectric point id the pH where the electric mobility is zero). Values were obtained by physical measurement of the distance from the center of the band to the edge of the anode end of the gel. The distance is converted to pI by calculations, assuming linear relationship between the two nearest points of the pH gradient.

RESULTS

The eye lens protein pattern of the hyprid of O. niloticus x O. aureus (Fig. 2 A) is characterized by three major bands at the pI 4.20 and 4.60 also by one minor band at the pI 3.75. While the pattern of the pure strain is characterized by the minor band at the pI 4.00 (Fig. 2 B).

The protein pattern of the pure strain of O. niloticus had been taken as a reference for the patterns of the fishes from different locations.

The protein patterns of all the five populations have a distinct bands at the pI 3.50, 4.20, 4.50, 4.75, 5.20, 5.50, 6.00, 7.00, 7.25, 7.75, 8.50, 8.80, 9.00, 9.40, 9.50 and 9.60 but few minor differences can be distinguished.

The band at pI 5.15 is more clear in the protein pattern of the fishes from Lake Nacer (Fig. 2 C). The band at pI 7.60 is relatively faint in the patterns of the fishes from both of Lake Naser and Aswan region (Fig. 2 C & D). Both of the two bands at the pI 8.70 and 9.60 are not very well presented in the pattern of the fishes from Cairo region (Fig. 2 E). On the other hand, the band at the pI 3.85 is more distinct in the pattern of the fishes from Lake Mariut than in other patterns (Fig. 2 F). Finally, the band at the pI 3.60 is distinct in the pattern of the fishes from Lake Manzallah than other patterns (Fig. 2 G).

DISCUSSION

Eye lens protein was chosen because of its purity. It is worth to mention, that all the fishes examined in the present study had the typical morphological characters of O. niloticus as the caudal fin distinctively marked with dark vertical bars and the dorsal fin having black or gray edge with 17 sharp dorsal spines. But in a free breeding population, the morphology and morphometry can nor guarantee the purity of Tilapia species necessary for hybridization purposes (Anonymous, 1967). Therefore, IEF was used to examine the purity of the population of O. niloticus in Egypt.

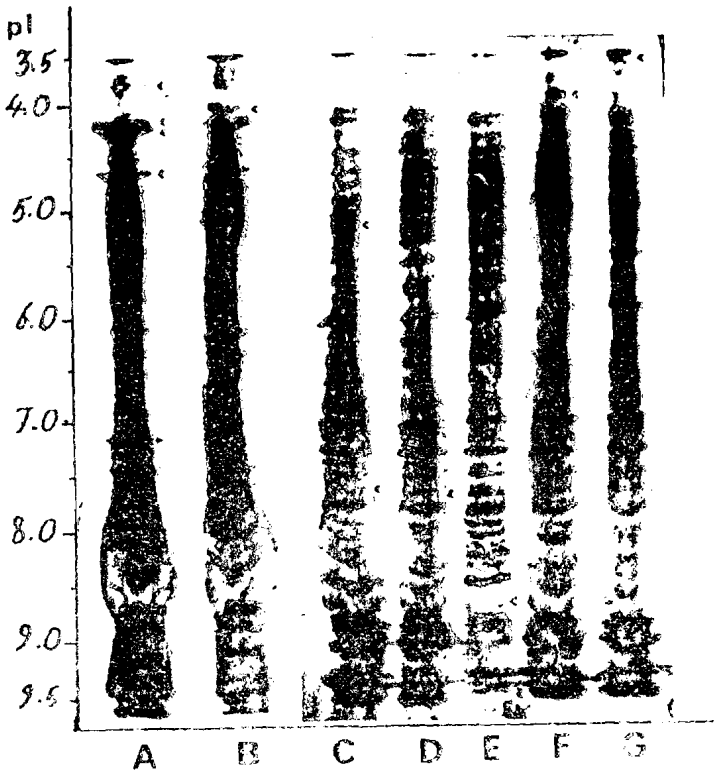

FIG .2.
General protein patterns separated in a PH gradient 3.5-9.5.
A. Hybid, B. pure strain, C. Lake Naser, D. Aswan region, E. Cairo region, F. Lake Mariut and G. Lake Manzallah.

The results obtained here revealed that only minor differences were observed between the patterns of the eye lens protein of the fishes from different habitats. Howevere, these differences would appear to be an artifact.

The protein patterns of the fishes from the five different habitats are similar to that of the pure strain which indicate a high degree of genetic similarity. Also these patterns differ than that of the hybride, which may indicate that no natural hybridization has been occurred.

According to Avtalion et al. (1976) each species of Tilapia has its specific protein. Okishima et al. (1984), Ng et al. (1986) and O'Maoileidigh et al. (1988) found that IEF was able to identify different populations within the same species.

Based on the results of IEF of the eye lens protein for the fishes from Lake Naser, Aswan region, Cairo region, Lake Mariut and Lake Manzallah, one can state that, Oreochromis niloticus in these water masses still at the present time has the proper protein characters of the pure strain.

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