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Elektroforetyczny rozdział na żelu poliakryloamidowym dehydrogenazy mleczanowej, transferyny i esteraz surowicy krwi siedmiu linii hodowlanych karpi (*Cyprinus carpio* L.)

Electrophoretic separation of blood serum lactate dehydrogenase, transferin, and esterases on polyacrylamide gel in seven carp breed lines (*Cyprinus carpio* L.)

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Abstract — Electrophoretic separation of lactate dehydrogenase, transferin, and esterases of seven carp breed lines on polyacrylamide gel was carried out. It was found that out of the investigated proteins, only esterases could be used to characterize the breeding materials, since in various lines they have different electrophoretic patterns.

The aim of the present work was to find out an easy method of distinguishing carps from various breed lines. Electrophoretic technique was used on the assumption that it might help in estimating the genetic patterns of the individual populations in a given species. The separation patterns of protein reflect its polymorphic forms which, as it is acknowledged, are directly related with the genotype. Therefore, the electrophoretic investigations frequently have higher taxonomic value than the biometric and meristic features (Dessaur et al. 1962, Manwell, Baker 1963, Manwell et al. 1967, Nyman, Westin 1969, Wilkins 1972).

Material and method

The investigation on the electrophoretic separation of lactate dehydrogenase, transferin and esterases of blood serum was carried out on

fishes belonging to seven carp families bred in the Experimental Farm of the Polish Academy of Sciences at Gołysz-Zaborze in the Upper Vistula river basin. Families Nos 2 and 3 are Polish carp bred since 1953 from a milter imported from the Mazurian Lake District and a spawner from the Gołysz-Zaborze Experimental Farm. These families differ by their selection trend. In the Course of the 25-year breeding period family No. 2 has been selected with respect to scaling and the highest individual growth against the background of the stock and now it is characterized by the typical scaling of the mirror carp, by great vitality and good growth. In family No. 3, also maintained by inbreeding, the selection has not been carried out with respect to scaling but to the reliability of growth rate and health. These carps are not typically scaled but they have regular body shape, good growth and good health. The third carp line, bred for years in the Gołysz-Zaborze Experimental Farm, is a Hungarian family of unknown origin (WA) with the mirror carp scaling type. It gives crossbreeds of good productivity with all Gołysz carp lines. Apart from the above-mentioned lines, the investigation included carps imported from Hungary in 1973, belonging to four breed lines: the Szeged variety of the mirror carp, No. 77, characterized by good weight increases, the Sumonyi ("88") variety of the mirror carp, though with irregular scaling, one of the best carps in Hungary, characterized by good weight increases, the "78" variety from a crossbreed of "77" × „88" families, and the Tólg family from the Bikal Experimental Farm in southern Hungary.

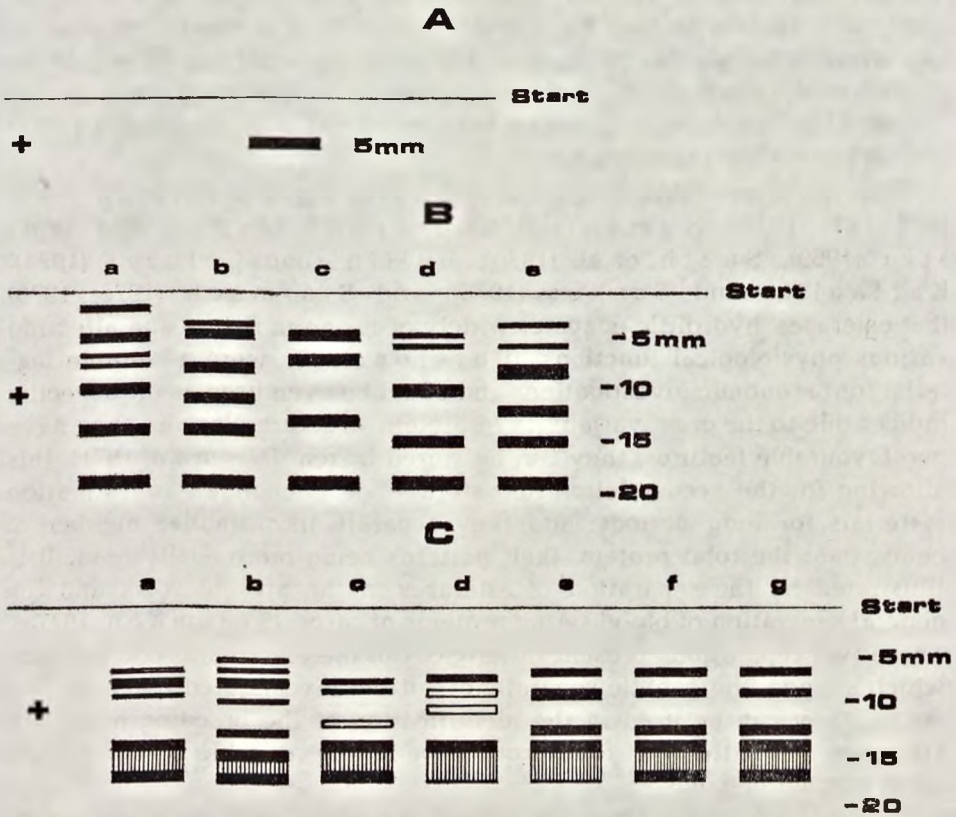
Blood samples from the anal vein were taken from 15 representatives of the above-mentioned families. They were treated with the solution used for preserving human blood, acidum citricum dextrosa (Aqua pro injectione 100 ml, sodium citrate 1.32 g, Glucose 1.47 g, Citric acid 0.48 g) in the ratio of 1 : 4, and transported at 0°C to the laboratory where the morphotic elements were separated by centrifugation at 18 000/min. for 5 minutes. The separated blood serum was frozen at —20°C. The disc electrophoresis was carried out on 8.5% polyacrylamide gel for 55 min. at the current intensity of 2 mA per 1 pipe, 6 mm in diameter and 75 mm in length, using the following pH 8.6 buffers: 0.004 M Tris, 0.000048 M citric acid, and 0.00032 M EDTA for gel, and 0.05 M H_3Bo_3 and LiOH 0.018 M for vessels. After the electrophoresis was completed, proteins separated on gel were stained as follows: lactate dehydrogenase according to Nyman (1967), esterases by the method described by Nyman (1970), and transpherins according to Mueller et al. (1962).

Results and discussion

In the investigated carps no differences were observed as far as lactate dehydrogenase was concerned (fig. 1A).

The transferins of the investigated fish form five electrophoretic patterns (fig. 1B—a, b, c, d, e). For the "Wa" family 3 patterns were found (fig. 1B—a, b, c), for the "3" family two patterns (fig. 1B—b, d), while for the families "88", "78", "77", and „Tólg", i.e. all Hungarian families imported in 1973, two patterns were observed (fig. 1B—d, e). Carps of the "2" family had one pattern only (fig. 1B—c).

Contrary to lactate dehydrogenase and transferins the blood serum enzymes of the esterase group have different separations in the investigated fish, except for 78 and 88 families, this being particularly visible



Ryc. 1. Elektroforetyczny rozdział na żelu poliakryloamidowym: A — dehydrogenazy mleczanowej surowicy krwi; B — transferyn surowicy krwi: a — "WA"; b — "WA", "3"; c — "2"; d — "88", "3", "77", "78" i "Tólg"; e — "WA"; "77", "78", "88" i "Tólg"; C — esteraz surowicy krwi: a — "2"; b — "3"; c — "WA"; d — "Tólg"; e — "77"; f — "78"; g — "88"

Fig. 1. Electrophoretic separation of blood serum lactate dehydrogenase (A); of blood serum transferin (B): a — "WA"; b — "WA", "3"; c — "2"; d — "88", "3", "77", "78" and "Tólg"; e — "WA", "77", "78", "88" and "Tólg"; and of blood serum non specific esterases (C): a — "2"; b — "3"; c — "WA"; d — "Tólg"; e — "77"; f — "78"; g — "88" on poliacrylamide gel

in the families "2", "3", "WA", and „Tólg" (fig. 1C—a, b, c, d). The patterns of "78" and "88" did not differ from each other (fig. 1C—f, g), as it was mentioned above, while the "77" pattern (fig. 1C—e) showed great similarity to "78" and "88" patterns, though it differed from them by the width of the second band in the first part of the pattern and by the absence of the furthest one in the second part lying between the 13—17 mm from the start. The „Tólg" family is also characterized by the absence of this band, but the first part of its pattern (fig. 1C—d, e) is different from that of "77". The "78" family is a crossbreed of the "77" and "88" families. All "78" individuals examined by me had the pattern shown in fig. 1C—f, identical with that of the "88" family. Probably, if a greater number of fish were included, the patterns of the other parental family would be encountered. However, the picture is obscured by the great similarity of "77" and "88" patterns, suggesting near relationship of the Szeged ("77") and Sumonyi ("88") families.

Thus the present investigation supported the views of Nyman (1965, 1967, 1971, 1972), Nyman and Shaw (1971), Nyman and Westin (1969), Smith et al. (1970), Bullini and Caluzzi (1974), Kartagilis and Tsekos (1975), and Starmach (1975, 1976) that esterases, hydrolitic enzymes widely occuring in nature and affecting various physiological functions (Burston 1962) were a suitable material for taxonomic investigations and could be even used as intraspecific indices due to the great variability of protein structure. Besides, they have two favourable features: they can be stored frozen (Nyman 1971), this allowing for the accumulation and storage of unchanged investigation materials for long periods, and they separate into smaller number of bands than the total protein, their patterns being more easily read. It is illustrated by the separation of esterases in the present work and the general separation of blood serum proteins of carps (Starmach 1977a). These two separations present differences in the electrophoretic patterns which support the genetic separateness of the investigated fish and, therefore, they can be used in the identification of the breeding materials. However, the patterns of total protein are hardly readable because of the great number of bands.

In investigations of this type besides the selection of the protein most suitable for taxonomic aims for a given species, an important factor is the use of a suitable electrophoretic method and, above all, of carriers characterized by certain separation capabilities, of buffers and current intensity. This is supported both by the present results and the separation of esterases on starch gel carried out by me in 1977 (Starmach 1977a). The separation of protein then obtained was poorer, the determination of families to which the investigated individuals belonged having been impossible.

STRESZCZENIE

Przeprowadzono rozdział elektroforetyczny na żelu poliakryloamidowym, dehydrogenazy mleczanowej, transferyny i esteraz surowicy krwi. Celem tych badań było opracowanie metody pozwalającej na łatwe odróżnianie od siebie 7 linii hodowlanych karpi (*Cyprinus carpio L.*).

Stwierdzono, że dehydrogenaza mleczanowa nie wykazuje u badanych karpi żadnych różnic. Transferyna tworzy 5 wzorów elektroforetycznych, z których 4 grupują wszystkie badane ryby z wyjątkiem jednej rodziny posiadającej odrębny wzór. Esterazy natomiast mają odmienny rozdział u poszczególnych rodzin karpi, mogą więc być wykorzystane do różnicowania materiału hodowlanego.

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