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Elektroforetyczny rozdział na żelu skrobiowym esteraz surowicy krwi, śledziony i wątroby dziewięciu rodzin karpi

Electrophoretic separation on starch gel of blood serum, spleen, and liver esterases in nine carp families

Wpłynęło 30 czerwca 1976 r.

A b s t r a c t — Electrophoretic separation of esterases on starch gel was carried out in nine families of carp selected with respect to scaliness and cultured for many years in line purity at the Experimental Farm of the Polish Academy of Sciences at Gołysz.

It was found that liver and spleen esterases in the investigated families did not show any differences. Polymorphous patterns of blood serum esterases differ from one another in the frequency of F esterases and in an additional band occurring in the pattern of one of the families.

Material and method

The aim of the present paper was to demonstrate and estimate the differences in protein structure in a number of carp families by means of electrophoretic separation of enzymes of the group of esterases present in the blood serum, liver, and spleen. The so-called carp families were selected with respect to scaliness and cultured for a long time in purity of lines at the Experimental Farm of the Polish Academy of Sciences at Gołysz.

The following families belong to them: R6 i.e. frame one from Poland, R3 — untypical from Ochaby, "Hungarian from Gołysz" (this means carp of Hungarian origin cultured at Gołysz for several generations), and the family from Knyszyn. Apart from the above-mentioned families investigations were also carried out on carp imported to the Gołysz Experimental Farm from Hungary in 1973 and denoted with the following

symbols: 77, 78, 88, and Tölg, and on carp cultured at the carp farm at Przemków in the Province of Wrocław.

After two weeks of acclimatization blood was taken from the anal vein, and samples from the liver and spleen from the fry of the mentioned carp. These materials were stored at a temperature of —20°C till the moment of carrying out electrophoretic separation.

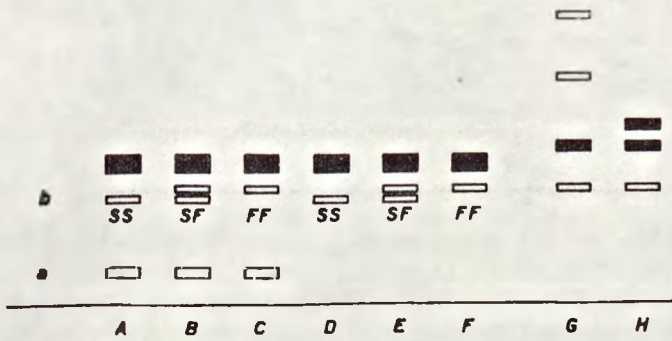
The electrophoretic separation of enzymes of the esterases group was carried out on a horizontally placed 10.55 per cent starch gel put on trays prepared according to the formula given by Tsuyuki (1963). A system of buffers according to Ashton and Braden (1961) was applied to the starch gel and electrophoretic vessels, only the molarity of the solution being changed from 0.181 M to 0.3 M. The time of electrophoresis was fixed for 1 hour and 50 minutes at a current voltage of 6.5 V per 1 cm of gel. Stains described by Nyman (1970) were used for staining the esterase enzymes.

Investigation results

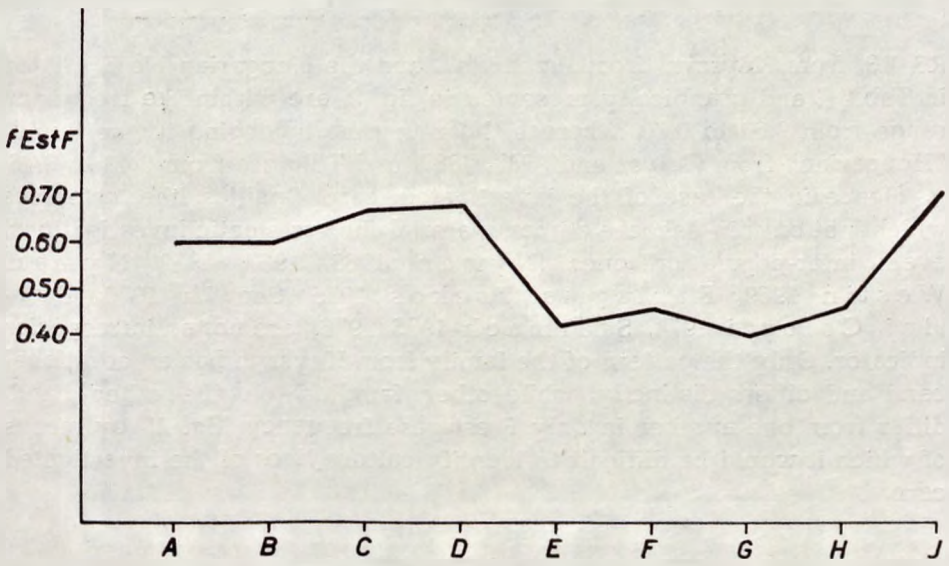
Patterns of the electrophoretic separation of liver and spleen esterases do not show any differences in the investigated families (fig. 1 G, H). Except the carp from Knyszyn, which have an additional "a" band in the pattern (fig. 1 A, B, C) the blood serum esterases are identical (fig. 1 D, E, F), showing in all investigated families (fig. 1 from A to F) in the zone marked on this figure with the letter b, a polymorphism which is common in the blood serum esterases in fish and has been frequently described (Nyman 1965, 1967, 1972, Nyman, Westin 1969, Starmach 1975 and others). In that part of the pattern they have three different arrangements of bands, occurring in individual families with various frequency. This phenomenon is caused by two allele which, in consequence of cross-breedings, form two homozygote patterns: "SS" (the band migrating slower to the anode) and "FF" (the band which migrates faster), and one homozygote pattern, "SF".

In the nine examined families of carp the observed frequencies of occurrence of individual polymorphous patterns show certain discrepancies in comparison with those calculated from Hardy-Weinberg's law. Only families R3, Hungarians from Gołysz, and lines 78, 88 imported from Hungary in 1973 behave in a different way. As a whole, however, the relation of frequency of the observed patterns to the expected ones is within the limits of significance (Table I), which indicates a certain genetic balance in these families.

Frequencies of F esterase occurrence in carp belonging to the family



Ryc. 1. Elektroforetyczne wzory esteraz surowicy krwi, wątroby i śledziony. A, B, C — esterazy surowicy krwi karpi rodziny knyszyńskiej; D, E, F — esterazy surowicy krwi następujących rodzin karpi: R6, R3, węgierskiej z Gołysza, Tölg, „77", „78", „88"; karpi z Przemkowa; G — wzór esteraz wątroby wszystkich badanych ryb; H — wzór esteraz śledziony wszystkich badanych ryb; a, b — objaśnienia w tekście; SS — homozygotyczny pasek wolno wędrujący; SF — heterozygotyczny wzór; FF — pasek szybko wędrujący
Fig. 1. Electrophoretic patterns of blood serum, liver, and spleen esterases. A, B, C — blood serum esterases in carp of the family from Knyszyn; D, E, F — blood serum esterases of the following carp families: R6, R3, "Hungarian" from Gołysz, Tölg, "77", "78", "88", carp from Przemków; G — pattern of liver esterases of all investigated fish; H — pattern of spleen esterases of all investigated fish; a, b — explanations in the text; SS — homozygotous band migrating slowly; SF — homozygotous pattern; FF — band migrating fast



Ryc. 2. Krzywa frekwencji esteraz F. A — R6; B — R3; C — Knyszyńskie; D — z Przemkowa; E — węgierska z Gołysza; F — „78"; G — „88"; H — Tölg; I — „77"
Fig. 2. F esterase frequency curve. A — R6; B — R3; C — from Knyszyn; D — from Przemków; E — "Hungarian" from Gołysz; F — "78"; G — "88"; H — Tölg; I — "77"

Tabela I. Wartości obserwowane i przewidywane wg prawa Hardy-Weinberga, frekwencja esteraz F oraz chi-kwadrat polimorficznych wzorców esteraz surowicy krwi badanych rodzin karpi

Table I. Observed and anticipated values according to Hardy Weinberg's law, frequency of F esterases and chi-square of polymorphous patterns of blood serum esterases in the investigated carp families

Rodzina Family	Liczba badanych ryb Number of investigated fish	FF	FS	SS	fF	X^2	Przybliżone prawdopodobieństwo Approximate probability
R6	20	9 7.2	6 9.6	5 3.2	0.60	2.62	0.20-0.30
R3	20	7 7.2	10 9.6	3 3.2	0.60	0.98	0.30-0.50
Knyszyńskie from Knyszyn	24	12 10.8	8 10.6	4 2.6	0.67	0.32	0.50-0.70
Węgry gołyskie Hungarian from Gołysz	20	8 8.4	10 9.2	2 2.4	0.42	0.33	0.50-0.70
77	26	15 13.0	7 10.7	4 2.1	0.71	0.56	0.30-0.50
78	23	5 4.8	11 11.5	7 6.7	0.46	0.11	0.70-0.80
88	25	6 5.3	11 12.5	8 7.3	0.40	0.18	0.90-0.95
Tölg	26	3 5.5	18 13.0	5 7.5	0.46	1.17	0.20-0.30
Karpie z Przemkowa Carp from Przemków	45	22 25.2	17 19.8	6 4.5	0.68	1.30	0.50-0.70

R6, R3, from Knyszyn, from Przemków, and the Hungarian No 77, listed in Table I and graphically presented in fig. 2, are within the frequency range from 0.60 to 0.70, whereas those in carp belonging to the family "Hungarian" from Gołysz and "78", "88", and Tölg are from 0.40 to 0.46.

Hence in the case of the investigated carp families the esterases which, not only in fish, are a good material for systematic investigations, even inter-population ones (Nyman 1965, 1967, 1972, Nyman, Westin 1969, Kartaglis, Tsekos 1975, Smith 1970, Bullini, Coluzzi 1974, Starmach 1975, 1976), are not an intraspecies indicator. Only the pattern of the family from Knyszyn has an additional band and differs distinctly, while other families, on the other hand, differ from one another in only F esterase frequency (fig. 2), by means of which it would be difficult to identify culture lines of the investigated carp.

STRESZCZENIE

Celem niniejszej pracy była próba wykazania i oceny różnic w strukturze białka. Za pomocą elektroforetycznego rozdziału na żelu skrobiowym enzymów z grupy esteraz zawartych w surowicy krwi, wątrobie i śledzionie dziewięciu rodzin karpi wyselekcjo-

nowanych pod względem ułuszczenia i hodowanych od wielu lat w czystości linii w Zakładzie Doświadczalnym Polskiej Akademii Nauk w Gołyszu.

Stwierdzono, że wzory elektroforetycznego rozdziału esteraz wątroby i śledziony nie wykazują u badanych rodzin żadnych różnic. Polimorficzne wzory esteraz surowicy krwi różnią się między sobą frekwencją esteraz F oraz występującym we wzorze jednej z rodzin dodatkowym paskiem.

Tak więc w przypadku badanych rodzin karpi, esterazy, pomimo że należą do enzymów będących nie tylko u ryb dobrym materiałem do badań systematycznych nawet międzypopulacyjnych, nie spełniają roli wskaźnika wewnątrzgatunkowego. Tylko bowiem wzór jednej z rodzin ma dodatkowy pasek i jest wyraźnie odrębny. Inne rodziny różnią się między sobą jedynie frekwencją esteraz F, przy pomocy której trudno by było zidentyfikować pojedyncze linie hodowlane karpi.

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