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FRACTIONING OF PROTEIN EXTRACTS OF SIX *TRICHINELLA* ISOLATES BY THE METHODS OF ISOELECTROFOCUSING AND SILVER STAINING

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Abstract

Comparative electrophoretical studies on protein extracts from six *Trichinella* isolates have been carried out with the purpose of obtaining novel data about their species characterization. The methods of isofocusing and silver staining have been used. The electrophoretical analysis of the water-soluble proteins of the six isolates: ISS03, ISS13, ISS10, ISS02, ISS029, ISS035, has yielded as a result a heterogeneity both of number and electrophoretical mobility of the individual protein fractions. On the basis of the obtained results as well as on the data from studies of other authors [1–3] the six isolates under study have been established to belong to the species *T. spiralis*, *T. pseudospiralis*, *T. nativa*, *T. britovi*, *T. nelsoni*, *T. murrelli*.

Key words: *Trichinella* species, isofocusing, silver staining

Introduction. The identification of the biochemical peculiarities of a given taxon related to its phylogenetic origins determines its precise place in systematics. The scientific systematics of a number of organisms cannot be accomplished only by using their morphological features. The studies on the biochemical speci-

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ficities of the different groups of organisms present a significant interest and constitute a separate subdivision of evolutionary biochemistry.

According to KLIMENKO [4], thorough biochemical investigations on the helminthes and especially on those of a dubious systematic position can help the precise determination of their taxonomy. For establishing the species differences accounting for the diversity or uniqueness of the metabolic processes, the study on the proteins appears to be the most rational one.

The method of electrophoresis in PAAG is a reliable biochemical method by which differences between the helminth species under study can be established and it is recommended as a diagnostic tool especially in cases of species whose individuality is disputable [5, 6].

According to certain authors [7,8] PAAE, isoelectrofocusing and silver staining represent an objective feature for the differential diagnostics of helminthes especially with the view that hosts do not exert influence on the protein composition of the studied helminth.

Material and methods. Trichinellae. For infecting the experimental animals and the obtaining *Trichinella* larvae the isolates: ISS03, ISS13, ISS10, ISS02, ISS029, ISS035 (courtesy of Dr. E. Pozio) from the World Reference Laboratory for Trichinellosis – Rome, Italy were used. The isolates were maintained by passaging in mice and rats.

Experimental animals and yield of* Trichinellae *from muscle mass. 180 white Balb/C mice (six groups of 30) were used. Each mouse was infected with 200 *Trichinella* larvae from the corresponding isolate. On day 50 from the beginning of the experiment the animals were sacrificed in accordance with the ethical norms for humane attitude towards the animals. Following the definitive digestion of the muscle mass by pepsin and hydrochloric acid, the *Trichinella* larvae were utilized for the production of the antigen.

***Antigens* (*total extracts from muscle* Trichinellae).** The suspensions of muscle Trichinellae in PBS (1:2) from the six *Trichinella* isolates were thrice homogenized in the glass homogenizer of Poter (the procedure is unique for each isolate). The supernatant following its protein content being defined after LOWRY et al., [9] was divided into small quantities which were kept at $-25\,^{\circ}$C.

Isoelectrofocusing. The method of analytical isoelectrofocusing [10, 11] was used for analysis of the water-soluble proteins of the six *Trichinella* isolates. For the implementation of the IEF, a separation chamber Multiphor, LKB was used and the ready plates with PAAG in a concentration of 5% and a pH range from 3 to 10 with a concentration of the ampholine of 2.4% and a size of 122/110/1 mm were the product of Pharmacia Fine Chemicals. 1M H_3PO_4 was used as anode buffer and 1 M NaOH – as a cathode one. The samples were placed in the middle of the plate on the paper starts in a quantity of 20 µl with a protein content of the antigens of 10 mg/ml. As markers we used ready-made kind of proteins with defined isoelectric points of the firm Pharmacia Fine Chemicals. The markers

were placed on paper starts at a distance of 6.67 cm from the cathode. The IEF was run at an initial voltage of 200 V and the last one reached up to 1200 V. The electrophoresis lasted for about 90 min. After fishing the electrophoresis, the gel was fixed for 1 h in a solution of trichloracetic acid and sulphosalicylic acid in methanol and distilled water. The staining was performed in a solution of Coomassie Brilliant Blue R – 250 for 10 min at 60 °C in water bath.

Silver staining. Thin-layer PAA-electrophoresis [10, 11] was carried out on an apparatus for vertical electrophoresis. The separating 10.5% PAAG polymerized between two glass plates of a thickness of 0.1 mm and a length of the run 12 cm. The electrophoresis was carried out in the course of 120 min at 4 °C and electric current of 40 mA/gel. As a coloured witness we used 2% solution of bromophenolblue. After the accomplishment of the electrophoresis the PAAG was processed and stained after the method of Zacharieva et al. [8].

Results and discussion. The thin-layer PAAG electrophoreses for isofocusing and silver staining have been carried out on apparatus for horizontal and vertical electrophoreses with the six *Trichinella* isolates being thrice investigated electrophoretically each. All phoregrams for the six *Trichinella* isolates showed a full reproducibility of the results from the separation of the water-soluble proteins.

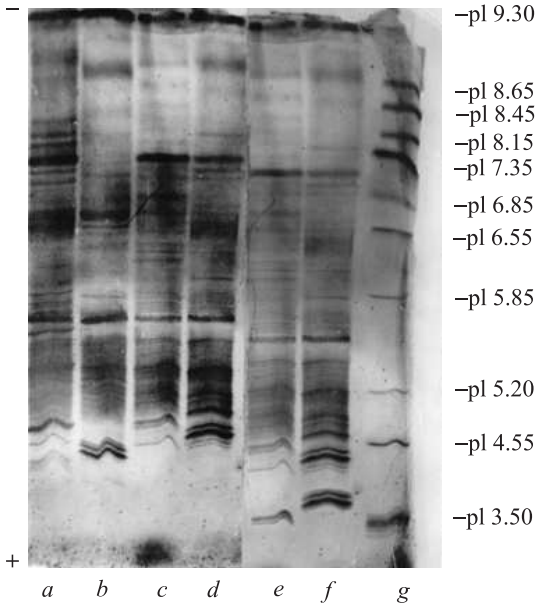

Fig. 1. Phoregrams of the isoelectrofocusing of the water-soluble proteins of six *Trichinella* isolates: *a*) ISS03, *b*) ISS13, *c*) ISS10, *d*) ISS02, *e*) ISS029, *f*) ISS035, *g*) protein markers of a defined pI as a witness

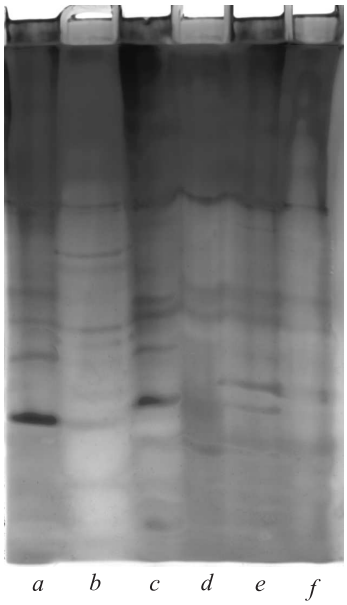

Fig. 2. Phoregrams in thin-layer PAAE of the water-soluble proteins of six *Trichinella* isolates stained with silver: *a*) ISS03, *b*) ISS13, *c*) ISS10, *d*) ISS02, *e*) ISS029, *f*) ISS035

Table 1

Protein fractions and the typical ones of a defined pI of the six *Trichinella* isolates following isoelectric focusing

Isolates	pH range		Total number of fractions	Typical fractions	pI
	acidic	alkaline			
ISS03	11	41	52	5	3.8; 6.5; 7.0; 8.8; 9
ISS13	10	32	42	3	4.3; 5.1; 8.3
ISS10	10	34	44	2	4.5; 4.7
ISS02	10	33	43	3	5.6; 6.4; 8.4
ISS029	11	30	41	2	3.5; 3.6
ISS035	10	34	44	3	3.75; 3.85; 4.1

The proteins and the other ampholytes display a typical pH value at which their overall charge is equal to zero. The samples from the water-soluble proteins placed in a medium with a pH gradient whose range coincides with the isoelectric points of the proteins are defined by their charges, pI of the individual proteins and the pH values at the spot of localization of the proteins. Upon applying an electric field through the solution, every charged protein will be directed in the pH gradient where pH is equal to its isoelectric point. The positively charged proteins take course towards the negative electrode (the cathode) and the negatively-charged ones – to the anode. This type of electrophoresis has been recently applied also for determining of the isoelectric points of mixtures of different proteins and their subunits in various species in helminthology.

The ampholine used bear ampholytes and was of constant of pI-values covering a pH range from 3 to 10. The division of the phoregrams in two zones – an acidic one and an alkaline one, is facilitated by the calibration sets of proteins with defined isoelectric points of the Pharmacia Fine Chemicals firm.

The phoregrams of the six *Trichinella* isolates under study are shown in Fig. 1 *a*–*g*).

The marker Myoglobin-acidic band (pI – 6.85) has been adopted as a borderline of the division of the acidic and alkaline range of pH. The analysis of the phoregrams of the water-soluble proteins under study has yielded a varying number of fractions. The isolate ISS02 is presented in the phoregram with 52 fractions, ISS13 – 42, ISS10 – 44, ISS02 – 43, ISS029 – 41, ISS035 – 44.

In Table 1 are shown: the number of fractions in the acidic and alkaline ranges, the total number of fractions, the typical fractions as well as their pI.

In the pH range from 3.50 to 6.85 the number of the fractions for the individual isolates is different.

The experimental data thus obtained (Table 1) show that each of the investigated isolates displays a specific spectrum of its water-soluble proteins. The results obtained are as follows: the different number of the protein fractions and

Table 2

Results from the thin-layer PAAE – 10.5% gel with silver staining of the water-soluble proteins of the six *Trichinella* isolates

Isolates	Zones			Fraction total	Typical fractions
	cathode	central	anode		
ISS03	1	4	3	8	2
ISS13	3	8	3	14	3
ISS10	2	5	4	11	3
ISS02	3	4	2	9	2
ISS29	3	4	3	10	2
ISS35	5	4	2	11	3

their different electrophoretic mobility as well as the different isoelectric points of defined typical fractions for every isolate give us grounds to identify the studied isolates as different species.

Silver staining – after the performed vertical thin-layer PAAE in a 10.5% gel, an incubation of the worked gel with the six *Trichinella* isolates has been carried out after the method of Zacharieva et al. [8]. These studies are carried out for the first time in parasitology and the possibility for this method to be used in the study and defining the systematic affiliation of the *Trichinella* genus representatives has been confirmed.

In Figure 2 are presented the phoregrams of the water-soluble protein fractions of the six *Trichinella* isolates stained with silver.

In Table 2 are presented the results from the total number of fractions as well as the typical ones for the six *Trichinella* isolates.

As a result of the established differences in the number of the protein components of each of the six *Trichinella* isolates understudy, different numbers of common and typical fractions have been established after silver staining. Their different electrophoretic mobility which is typical of each isolate fractions gives us grounds to admit that the method of Zacharieva et al. [8] could be successfully used in the *Trichinella* genus representatives for identification of isolates and in other experimental models in parasitology [12, 13].

The different numbers of the polypeptide fractions, the different electrophoretic mobility, different pI, as well as the studies of Pozio et al. [14] give as grounds to assume that the water-soluble proteins of the isolates: ISS03, ISS13, ISS10, ISS02, ISS029, ISS035, belong to the species: *T. spiralis*, *T. pseudospiralis*, *T. nativa*, *T. britovi*, *T. nelsoni* and *T. murrelli* and display both common protein components as well as ones typical of the individual isolates and can be successfully implemented in the identification of various strains and isolates from the *Trichinella* genus.

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