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# STUDIES OF PROTEINOGRAMS IN DERMATOPHYTES BY DISC ELECTROPHORESIS. I. PROTEIN BANDS IN RELATION TO GROWTH PHASE AND NITROGEN SOURCE.

P. Danev, E. Friedrich, V. Balabanov


Translation of "Izsledvaniya vurkhu proteinogramite pri dermatofitite s pomoshtta na diskovata elektroforeza. I. Proteinovite ivitsi v zavisimost ot fazata i iztochnika na azot" Dermatologiya i venerologiya (Sofia), Vol. 19, No. 2, 1980, pp. 82-85.



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16. Abstract

Homogenates were prepared from various growth phases of Microsporum gypseum grown on different amino acids as the nitrogen source. When analyzed on 7.5% polyacrylamide disc gels, the water-soluble proteins in these homogenates gave essentially identical banding patterns.

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# STUDIES OF PROTEINOGRAMS IN DERMATOPHYTES BY DISC ELECTROPHORESIS. I. PROTEIN BANDS IN RELATION TO GROWTH PHASE AND NITROGEN SOURCE

P. Danev, E. Friedrich, V. Balabanov[1]

Dermatophytes are among the most narrowly specialized fungi on /82* man and animals. The physiological manifestations of their pathogenicity are based on the fact that they are keratinophilic. As opposed to saprophytic fungi, they are capable of attacking intact, native keratin in vivo.

There are a variety of difficulties causing the deficit, even today, of a natural system of dermatophytes, and especially their pathogenic representatives, which comprise the majority. First we have their morphological similarity (e.g., convergence), resulting from the close conditions of their existence. Also important is their simplified morphology in parasitism, e.g., morphological reduction. We have found yet another physiological and biological reduction with parasitism, which, together with the specified morphological alterations during biological differentiation, complicates diagnosing the pathogenic strains. We should note here the lack of antigen test specificity and the inability to make a genetic determination.

Despite these limitations, the general characteristics of dermatophytic species make possible a single classification, close to natural, which is useful in epidemiology and the battle against dermatomycoses. This classification was achieved through a complex determination of the quality of the pathogenic species, by a combination of all diagnostic signs -- ecological and biological, in connection with the variability and dimorphism, versus ontophylogenetic,

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*Numbers in the margin indicate pagination in the foreign text.

parasitologic, and, not least of all, biochemical-enzymatic [2, 3, 6].

Disc electrophoresis, the subject of this series of studies, is /83 of particular interest for investigating the relationships between dermatophytes and explaining their taxonomy.

Disc electrophoresis on polyacrylamide gels is a method first introduced at the beginning of the 1960's by Davis and Ornstein [9, 10, 11, 12, 17, 18]. It is used to separate electrically charged biological macromolecules, such as proteins and nucleic acids. The separation is accomplished on one hand by molecular weight, on the other according to charge in an electric field. The molecular sieve effect depends on the gel's pores. Distinct protein fraction separation is achieved with this method.

We used this method to separate water-soluble mycelium proteins from various dermatophytes consisting of keratinophilic fungi, and used them to compare the electrophoretic patterns of the various dermatophytes.

Since corresponding studies have established a relationship between dermatophyte enzyme activity and growth phase, as well as nutrient medium nitrogen source, we also set out to establish whether or not dermatophyte protein fractions are affected by these parameters.

The results of our preliminary studies will be described below, while the protein band locations of the various dermatophyte species will be given in another publication.

Method. As the experimental fungus we used strain No. 243 of Microsporum gypseum, isolated in 1961.

Culture conditions. The preliminary culturing method of preparing the suspension of the experimental fungus has been described in previous publications [4, 5, 6, 6a].

The nutrient medium (culture medium) was a solution consisting of salts, amino acids, and glucose in the following composition:

| | |
|---|---|
| NaCl | 0.585 g/ℓ |
| $KH_2PO_4$ | 0.136 g/ℓ |
| $K_2HPO_4$ | 0.087 g/ℓ |
| $MgSO_4 \cdot 7H_2O$ | 0.247 g/ℓ |
| $CaCl_2 \cdot 6H_2O$ | 0.216 g/ℓ |
| Glucose | 9.910 g/ℓ |

The nitrogen source was varied as follows:
Series 1. One liter of nutrient medium contained glutamic acid (0.294 g), lysine (0.182 g), leucine (0.262 g), phenylalanine (0.330 g). The total nitrogen content per liter was 0.14 g.

Series 2. The nutrient medium contained double the amount of nitrogen, 0.24 g/ℓ, consisting in equal parts glutamic acid and cystine.

Series 3. One liter of culture medium contained 0.14 g of nitrogen, consisting entirely in glutamic acid.

The initial acidity (pH) of each nutrient medium was 4.8. After autoclaving it fell to 4.0.

200 ml Erlenmeyer flasks were filled with 50 ml of nutrient medium. After 20 min of autoclaving in these flasks at 120°C, 0.5 ml of fungus culture suspension was pipetted out, providing 0.08 g of mycelium dry weight per flask.

The tests were completed by reading the results at room temperature.

Mycelium processing and handling. The dry weight of material contained in 3 (three) flasks was determined on the day of the experiments. Mycelia from two or three other flasks were used to obtain the cell contents.

Dry weight determination. A fine-porosity paper filter, 90 mm in diameter, of a known weight was placed in a Buchner funnel with an internal diameter of 90 mm, after which the flask contents were poured over the filter. A water-jet pump was used to draw out the liquid, then the mycelia were washed with 5 ml each of buffer solution (pH 8.0). The filtrate and washed mycelium were placed in /84 a previously weighed Petri dish filled with two ml of acetone, then left three hours in an air-ventilation chamber.

The mycelia were then dried for 16 hours at 95°C. After cooling to room temperature, dry weight was determined. The mycelia were opened, using a previously described method [4, 5, 6, 6a], to obtain the cell contents. Lowry's method was used to determine the soluble part of the cell homogenate. The amount containing 50 micrograms of protein was determined by disc electrophoresis.

Three parallel flasks with mycelium, designated a, b, and c, were prepared for each experiment. Then identical samples of mycelium homogenate from the same flask were designated with the same letters. The experiments done on different days were sequentially numbered for each series.

Disc electrophoresis conditions. 7.5% polyacrylamide, containing 2.6% N,N'-methylene-bis-acrylamide and kept in test tubes, served as the matrix.

50 micrograms from the upper mycelium homogenate residue were pipetted over the gel. The pH of the separation medium was 9.5, and the pH of the buffer solution was 8.3; 220V and 4-5mA of current were applied to the test tubes. The fractions were fixed on the gel with 12% trichloracetic acid and stained with brilliant blue G-250 (in 12% trichloracetic acid). Clear, narrow bands resulted.

## Results

The mycelium dry weight values are presented in supplement 1, and the growth curve is given for M. gypseum.

From it we can see that fungi are present in the three experimental series at the first study, i.e., five days after culturing, in a logarithmic growth phase. The end of the logarithmic phase was reached in the first series after 15 days, in the second after 14 days. Since research on the last two series was done until the 23rd day of culturing, it was impossible to determine the end of the logarithmic phase for the third series, with glutamic acid as the nitrogen source, in which growth was slower.

Supplements 2 to 7 illustrate the proteinograms from series (1 and 2, M. gypseum) in the early logarithmic phase (5th day of study), late logarithmic phase (15th and 11th days, respectively), and in the stationary, corresponding to autolytic phase (33rd day for series 1, 23rd day for series 2). The good reproducibility of the results can be seen first of all in each figure. The mycelium homogenates from the second and third flasks (a, b, c) show the same banding patterns as the two tests of the same homogenate.

Comparison of the pherograms from the different growth phases established relatively corresponding protein banding for both series 1 (supplements 2, 3, and 4) and series 2 (supplements 5, 6, and 7). A considerable qualitative difference is seen with one protein moving rapidly under the conditions of our studies. This appears especially clearly in the autolytic phase (first band in figs. 4 and 7, but slightly or very slightly in the logarithmic phase -- figs. 2 and 3). It was necessary to see if these results could have been reproduced.

Supplement 2 and [illegible] from series 1 (glutamic acid, glycine, leucine, phenylalanine, and lysine) and supplements 5 to 7 of series 2 (glutamic acid, cystine) serve as a comparison of protein fractions from M. gypseum grown on various nitrogen sources. Further-

more, fig. 8 shows the protein bands from series 3 at the beginning and fig. [illegible] during the logarithmic phase after growth of M. gypseum with glutamic acid as the nitrogen source.

A qualitative difference is seen with the slowly moving proteins (bands between one and two cm). After growth with glutamic acid and cystine (series 2) these bands were more clearly distinguished in each of the five studies than in series 1 and 2. The minor qualitative differences result to some extent from the photographic reproduction. Thus we can state that the M. gypseum protein bands do not display major differences at different growth phases with differing amino acids as a nitrogen source. They agree strongly in each figure.

## Generalization

Disc electrophoresis was used to separate water-soluble proteins from an M. gypseum mycelium homogenate at various growth phases and after growth with differing amino acids as a nitrogen source. The obtained pherograms are very much the same and reveal no substantial differences.

References are given at the end of the second report.[2]
(References are contained on pp 86-89 of this publication).

2. We wish to express our special gratitude to Charlotte Rikus and Ingrid Zernal for their technical assistance, to Berbel Daneva for preparing the figures, and to Ingrid Senger for the photographs.