POLYACRYLAMIDE GEL ELECTROPHORESIS PATTERNS OF SOME IRANIAN MICROSPORUM AND TRICHOPHYTON SPECIES

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Abstract - The protein profiles of different Iranian clinical isolats of dermatophytes (Microsporum and Trichophyton) were compared by SDS polyacrilamide gel electrophoresis. Although some of the protein patterns were common in both genuses, there were some genus specific protein profiles. Also within the species variety of distinct protein were profiles obtained. Protein patterns can therefore distinguish between different genuses, species and strains.

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INTRODUCTION

The dermatophytes comprise a group of fungi of medical and veterinary importance which produce diseases essentially limited to the skin , hair and nails. Classification was originally based on a combination of clinical aspects of the disease and morphological characteristics of the fungi (1). The perfect state of several dermatophytes has been identified and these species have been reclassified from fungi imperfect to Ascomyceies.

Variety of techniques are available for the subspecific differentiation of strains of bacteria but few are readily applicable to fungi, especially filamentous fungi. SDS - PAGE to taxonomy has provided a valuable tool for the study of phylogenetic relationships. Protein structure is primarily an expression of the genotype of organism. Since species are determined by their genotypes, the study of proteins by electrophoresis is important in understanding of species. Homology of protein fractions as exhibited by common electrophoretic mobilities has been used by Ibrahim and coworkers (2). as a criterion of genetic affinity among taxa.

Jones and Noble (3) had investigated the epidemiology of M.canis infections by studing isoenzyme patterns and whole cell protein patterns of clinical isolates derived by PAGE.

Later Tucker and Noble (4, 5) reported on the use of PAGE protein patterns as a means of distinguishing between isolates of this fungal species.

This study presents comparative studies using PAGE of proteins from Iranian isolates of Microsporum canis (typical and atypical strains), M.distortom, Trichophyton mentagrophytes, T.rubrum, T.Verrucosum.

MATERIALS AND METHODS

Strains

Clinical isolates of M.canis typical and atypical strains (ten of each), M.distortum (1), reference strain NCPF 352, T.mentagrophytes (10), T.rubrum (9), T.verrucosum (9) were used.

Preparation of Cytoplasmic Extracts

Each isolate was subcultured on freshly prepared glucose/ peptone agar medium (glucose 20 g/L peptone 10g/L; agar 12 g/L). Cultures were incubated at 28°C for two weeks. When the fungal thallus covered approximately half of the agar surface the mycelium was scraped and transferred to universal bottles containing 10 ml of sterile glucose peptone broth and a few 5mm diameter

glass beads.

The lids were screwed down tightly and the contents homogenised using a vortex mixer. The mycelial suspensions were immediately transferred to two 500 ml conical flasks; each containing 150 ml of sterile glucose/peptone broth and incubated at 30°C in a shaking water bath. Incubation was continued until the mycelial growth almost completely filled the medium. The mycelium was then harvested by filtration/washed twice with sterile distilled water, weighed and frozen at -40°C until processed.

The defrosted mycelial mat was suspended in phosphate buffered saline pH:7.2 and distrupted in an E.L.M. 2500 homogeniser for eight periods of 1 min separated by 5 min cooling. The chamber was cooled by and outer Jacket containing an ice/water mixture. The extract was separated from the debris by centrifugation at 2500 g at 4°C, followed by 2500 g for 30 min at 4°C. The supernatant was removed, dialyzed for 24h at 4°C against repeat changes of distilled water and freeze dried.

Polyacrylamide Gel Electrophresis

Initially the protein content of sample of each cytoplasmic extract was determined using the Bradford method (6).

The extracts were disolved in minimal amount (0.5 mg freeze dried extract in 500 μl) of double - strength loading buffer (0.125 mol Tris - Hcl containing 2% sodium dodecyl sulphate, 2% mercaptoethanol, 10% glycerol, 2% bromophenol blue). The extract heated to 100°C for 2 minutes.

Volumes of 10μl containing 3μg protein were electrophoresed in 10% polyacrylamide gels. The method was that described by Laemmli (7) and Zaini and coworkers (8). with a stacking gel buffer of 0.125 mol Tris/HCl pH:6.8, a separating gel buffer of 0.375 mol Tirs/HCl pH:8.8 and tank buffer of 0.025 mol Tris/0.192 mol glycine pH:8.3.

The experiment was run at a constant current of 15 mA per gel through the stacking gel and 10 mA through the separating gel in an LKB Electrophoresis unit ESP 500/400 (Pharmacia, S-75182 UPPSALA, Sweden) and tube system (Titan plus Electrophoresis, power supply Helena). Reference standards in the molecular weight range 14000 to 95000 D were included on each gel to facilitate comparison of the obtained bands. When the tracking dye reached the bottom, the gel was removed and stained for 30 minutes in 0.5% coomassie blue R dissolved in methanol; water; glacial acetic acid solution 5:4:1, then destained in the same solvent until background was clear.

The patterns were scanned by using a densitometer (Helena, process - 24) fitted with a 590 nm filter. The molecular weights of proteins were calculated by determining their relative mobilities in 10% SDS-PAGE gel and comparing them with those of the molecular weight standards.

RESULTS

Clinical isolates of dermatophytes from Iranian and reference strain NCPF 352 were included in this investigation. A characteristic electrophoretic pattern of distinct protein fractions was obtained from the mycelial extracts. Similarities and differences were observed among the preparations from various species in the electrophoretic mobilities of the fractions.

The protein extract of typical strains of M. canis yielded 15, atypical strain of M. canis 17, M. distortum 17, NCPF 352; 18, T. mentagrophytes 13, T. rubrum 11, T. Verrucosum 15 fractions.

There was a similarity between protein patterns obtained from Microsporum and Trichophyton genus which have a total of four homologous fractions (Rf: 0.05, 0.57, 0.61, 0.77).

The protein profiles (Rf: 0.71, 0.85) exclusively found in Microsporum genus and protein fractions (Rf: 0.15, 0.74) were Trichophyton specific (Figs. 1, 2) M.distortum was distinguished from M.canis (typical, atypical, NCPF 352) by lacking the fraction (Rf: 0.264).

T.mentagrophytes was differentiated from T.rubrum and T.verrucosum by protein fractions (Rf: 0.19, 0.32, 0.59).
T.rubrum was distinguished from T. mentagrophytes and T.verrucosum by lacking the fraction (Rf: 0.82). T.verrucosum was identified from T.mentagrophytes and T.rubrum by fractions (Rf: 0.29, 0.36, 0.46). The similarities and differences among the patterns are summarized in Fig. 1.

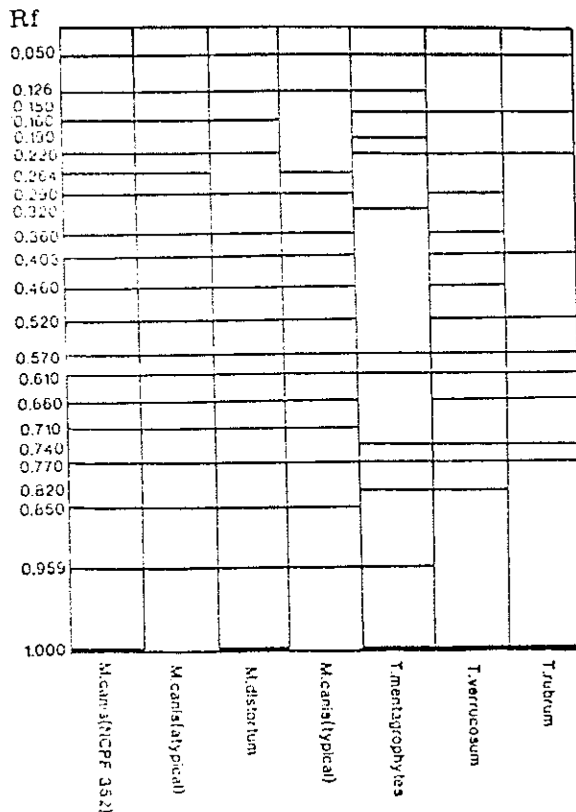

Fig. 1. Diagramatic comparison of presence or absence of protein fractions observed

DISCUSSION

Comparison of protein patterns may be regarded as an indirect method for comparison of microbial DNA, differences in genome being reflected in the structure of the encoded macromolecules. Good correlation has been found by Jackman and Pelcynska (9) between the results of DNA-DNA hybridization and polyacrylamide gel electrophoresis in a number of bacterial genera. The densitometric recording of total protein paterns and computer analysis allows the production of dendograms showing the overall percentage similarity between different isolates and has been used successfully in taxonomic studies of various bacteria (9, 10) and some fungi (2, 3). Of the few reported studies on dermatophytes which have used electrophoretic techniques, most have shown that the protein patterns obtained are species specific. Jones and Noble (3) revealed species specific isoenzymes of some dermatophytes which allowed some species to be recognized despite morphological variation.

Krempl - Lamprecht and Coworkers (11) used isoelectricfocusing to fractionate water - soluble proteins from 13 Microsporum species and found that the patterns obtained were species specific. Whilst Jeffries and Coworkers (12) used the same technique to differentiate between tester strains of M. canis (A. otate).

Tucker and Noble (4) examined native protein patterns of wild type and reference M.Canis (A.otae) stranins and suggested that clinical isolates have a common clonal origin.

Although the isolates of Microsporum and Trichophyton did form common fractions (Rf: 0.05, 0.57, 0.61, 0.77), the protein fractions which (Rf: 0.71, 0.85) exclusively found in Microsporum and fractions at (Rf: 0.15, 07.74) were Trichophyton specific. All the Microsporum strains lacked the fractions (Rf: 0.15, 0.19, 0.32, 0.74, 0.82).

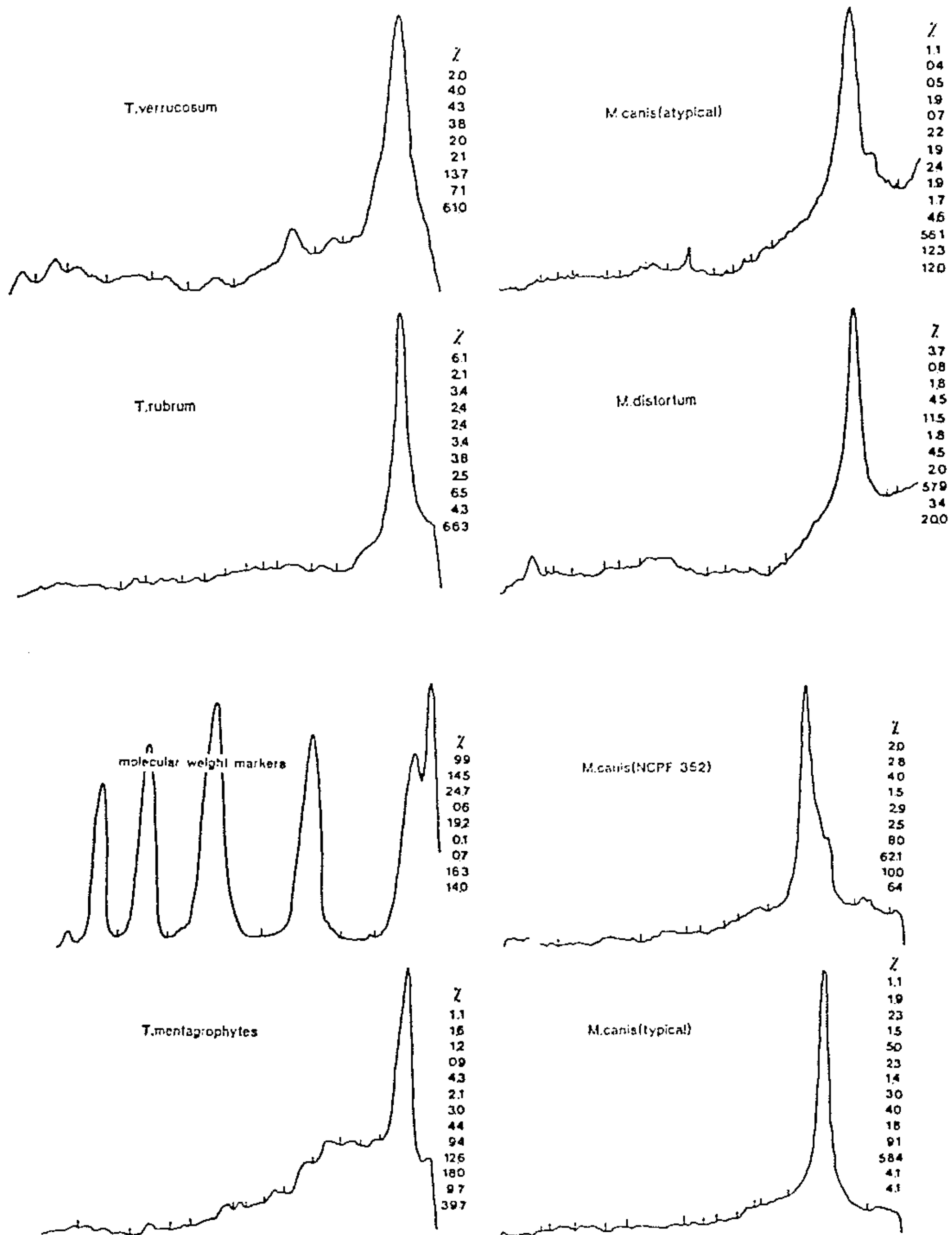

Fig. 2. Densitometer traces of protein patterns of microsporum and trichophyton species

While Tucker and Noble (5) reported that M.distortum was found to be indistinguishable from M.canis, in the present study M. distortum was differentiated from the other strains of M. canis by absence of the fraction (Rf:0.29). Moreover Iranian isolates of M.canis showed similarities to the NCPF 532 in the fractions at (Rf: 0.264, 0.71, 0.85).

Atypical strains of M.canis did not form the protein fractions (Rf: 0.16, 0.22).

The result of this study in general is similar to that of other investigations (11, 13) and indicates that the patterns obtained by SDS-PAGE are species specific and genous specific.

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