Iranian J. Publ. Health, Vol. 30, Nos. 1-2, PP. 71-72, 2001

The Protein Profiles of *Trichophyton rubrum* by SDS-PAGE

***M Mahmoudi Rad[1], F Zaini[1], M Pezeshki[1], P Kordbacheh[1],
E Elmi Akhouni[2], M Emami[1]***

[1]*Dept. of Medical Mycology, School of Public Health and Institute of Public HealthResearch, Tehran University of Medical Sciences, P.O.Box 14155-6446, Tehran, Iran.*
[2]*Dept. of Biochemistry, School of Medicine, Tehran University of Medical Sciences, P.O.Box 14174, Tehran, Iran.*

Key Words: Trichophyton rubrum, dermatophyte, SDS-PAGE

ABSTRACT

In the present study, we investigated total cell protein patterns of ten isolates of Trichophyton rubrum by SDS-PAGE on 12.5% polyacrylamide resolving gels. Twenty-two protein bands were detected with molecular weights in the range of 23.2 to 131.8 KD. Proteins of 23.2, 25.4, 26.7, 28.2, 30.2, 33.1, 34.7, 38,41.7, 47.9, 52.5, 56.2, 61.7, 67.6, 70.8, 75.9, 80, 84.1, 93.3, 102.3, 114.8, 131.8 were present but their frequencies varied among the isolates. Protein bands of 23.2, 38, 47.9, 52.5, 84.1 were common among the isolates and could be specific to recognize species differences. Protein analysis by SDS-PAGE could be considered a useful technique in identifying differences among the dermatophyte isolates.

INTRODUCTION

The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair and nails) of humans and other animals to produce an infection, dermatophytosis, commonly referred to as ringworm. They are classified in three anamorphic genera, *Epidermophyton, Microsporum* and *Trichophyton.* The vast majority of chronic dermatophyte infection of human skin are caused by *Trichophyton rubrum* (7). The study of proteins by electrophoresis for taxonomy has provided a valuable tool for the study of phylogenetic relationships. Homology of protein fractions by electrophoretic methods has been used in some studies previously (3). In the present study we describe the protein patterns of two reference strains and eight Iranian strains by SDS-PAGE.

MATERIALS AND METHODS

Growth of Microorganisms

Eight clinical isolates and two reference stains (NCPF325 and NCPF420) were used. Each of them was subcultured on freshly prepared Sabouraud dextrose agar and incubated at 28°C for 2 weeks. The mycelia were scraped and transferred to universal bottles containing 10 ml sterile Sabouraud dextrose broth 2% and a few 5 mm diameter glass beads. After shaking them to make a homogenized suspension, they were transferred to four 500 ml conical flasks; each containing 150 ml of sterile Sabouraud dextrose broth 2% (pH 5.5), and incubated at 28°C in a shaking water bath for 10 days. The fungi were killed by the addition of thimerosal to a final concentration of 0.02% W/V and the flasks left at 4°C overnight. The global shape mycelia was harvested by filtration, washed three times with sterile distilled water and stored at -40°C until processed.

Preparation of Cytoplasmic Extracts

Ten grams of defrosted mycelial mat was suspended in 10 ml of 0.14 mol phosphate buffered saline (PBS) pH 7.4, containing a cocktail of water or ethanol soluble protease inhibitors (benzamadine hydrochloride 10 micromol; N-ethyl maleimide 1mM; ethylamine diamine tetraacetic acid 1mM; bestatin 1mg/lit; phenyl-methyl-sulphonyl fluoride 1mM; tosylamino-2-phenylethyl chloromethyl ketone 0.1mM; pepstatin A 0.1 micromol, [Sigma]), and transferred to a homogenization flask (Edmund Buhler, Germany) to be homogenized. In order to tease them completely, they transferred to homogenization flasks containing 50 grams of glass beads (of 0.45 mm diameter). The extraction was performed in a MSK Braun Mill (F.T. Scientific Instrument Ltd., Germany) at 2800 rev/min for 6 subsequent periods of 30 seconds using CO_2 as a coolant. The homogenate was cleared of debris by centrifugation at 2500 g for 15 minutes at 4°C. Thereafter, the supernatant was centrifuged at 25000 g at 4°C for 30 minutes. The supernatant was removed, dialyzed for 24h at 4°C against repeat changes of running tap water and then freeze-dried (VIRITIS Company Gardiner, New York) (8).

SDS-PAGE

The protein content of each of samples was determined by the Bradford method (1). The extracts were disolved in an equal volume of double strength loading buffer (0.125 mol Tris-HCl containing 2% SDS, 2% 2ME, 10% glycerol, 2% bromphenol blue), and heated to 100°C for 2 minutes and finally centrifuged (13000 RPM for 5 min). Volumes of 10-20 microlitre, containing 3-10 micrograms protein were electrophoresed in 12.5% polyacrylamide gels.

The methods was based on that described previously (4) with a stacking gel buffer of 0.125 mol Tris-HCl, pH 6.8, a separating gel buffer of 0.375 mol Tris-HCl, pH 8.8, and tank buffer of 0.025 mol Tris-HCl, 0.192 mol glycine pH 8.3. The SDS-PAGE was run at 5 mA through the stacking gel and 7 mA through the separating gel in a Akhtarian Electrophoresis Unit, cooled by tap water, until the tracking dye had reached the bottom of the gel. Low molecular weight marker (Pharmacia, Sweden) were electrophoresed in parallel. The gel was removed and stained for protein with Coomassie Brilliant Blue R-250 (Sigma Chemical, USA) and destained in 40 % methanol, 10 % acetic acid (all v:v) in water (6,8). Protein

*Corresponding author, Tel:+98-21-8951583; Fax: + 98-21-6462267; E-mail: tavalaie@ut.ac.ir

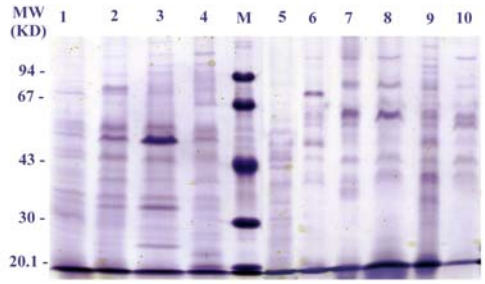
patterns were analyzed using a scanner (Genius), printed, and their molecular weights were calculated by comparing them with those of the standards.

RESULTS AND DISCUSSION

The cytoplasmic extracts complexity of *Trichophyton rubrum* was revealed by SDS-PAGE. About 22 clearly detectable mycelial protein bands over a wide range of molecular weight 23.2 to 131.8 KD were recognized (Fig.1). The best results were obtained with freshly prepared extracts. Proteins of: 23.2, 25.4, 26.7, 28.2, 30.2, 33.1, 34.7, 38, 41.7, 47.9, 52.5, 56.2, 61.7, 67.6, 70.8,75.9, 80, 84.1, 93.3, 102.3, 114.8, 131.8 were present but their frequencies varied amongthe isolates. Protein bands of 23.2, 38, 47.9, 52.5, 84.1 were common among the Iranian and reference isolates and could bespecies-specific.

The traditional taxonomy of the dermatophytes is based on gross and microscopic morphology with minor emphasis on physiology and nutrition. However, identification of isolates has been complicated by their overlapping characteristics, variability and pleomorphism. A variety of chemotaxonomic methods have been developed to bypass the traditional methods of identification and to determine relationships between the various species (7).

Fig. 1. **Protein profiles of ten isolates of *T.rubrum* on 12.5% SDS-PAGE**



(M = molecular weight marker, 1 and 5 = reference strains, and the others are Iranian isolates).

Cytoplasmic extract analysis by SDS-PAGE, one of the developed methods, for dermatophytes has not been adequately studied, possibly because of difficulties in obtaining a sufficient concentration of soluble proteins. However, by using the newly methods, the cytoplasmic extract preparation was successful. Longer homogenization periods in the presence of proteinase inhibitors enabled us to get sufficient concentrations of cytoplasmic proteins (2).

Comaprison of protein patterns may be regarded as an indirect method for comparsion of microbial DNA, differences in genome being reflected in the structure of the encoded macromolecules (8).

Of the few reports on dermatophytes which have used electrophoretic techniques, most have shown that the protein patterns obtained were species-specific (3, 5, 6).

REFERENCES

1. Bradford MM (1979): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, **22**: 248-54.
2. Garg AP and Muller J (1991): Preparation of antigens from Trichophyton mentagrophytes using a new semi-solid culture medium and their characterization by SDS-PAGE and immuno-logical techniques. *Mycoses*, **35**(3): 34955
3. Ibrahim GO, Bievre C, Romain F and Letoffe S(1985): Comparative electrophoresis isoelectro-focusing and numerical taxonomy of some isolates of Fonseca pedrosoi and allied fungi. *J Med Vet Mycol*, **23**: 253-64.
4. Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**: 680-85.
5. Tucker WD L and Noble WC (1990): The value of electrophoretic protein patterns for the study of Microsporum canis. *J Med Vet Mycol*, **28**: 117-23.
6. Tucker WD L and Noble WC (1991): Polyacryl-amide gel electrophoresis patterns of some *Microsporum* species. *Mycoses*, **34**(6): 303-7.
7. Weitzman I and Summerbell RC (1995): The dermatophytes. *Clin Microbiol Rev*, **8**(2): 240-59.
8. Zaini F, Moore MK, Hathi D, Hay RJ and Noble WC (1991): The antigenic composition and protein profiles of eumycetoma agents. *Mycoses*, **34**: 19-2