

Investigation of *Trichophyton verrucosum* proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

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Trichophyton verrucosum is one of the most common dermatophyte that parasitizes keratinized tissues in human and animal. This study compares the proteins of 5 varieties of *Trichophyton verrucosum*, which obtained from human and animals. At first, they were cultured on the specific media separately and incubated at 37 °C for one month. For preparation of protein extracts, the colonies of fungi were transferred to Sabouraud's liquid medium and the amount of proteins was determined by Bradford method after growth of fungi. SDS-PAGE with 13% polyacrylamide separating gel and a discontinuous buffer system was carried out for protein analysis. Coomassie blue G250 was used for gel staining and different bands were appeared. Gel was scanned with a Helena densitometer for protein patterns. Fourteen protein bands with different molecular weights between 12730-92250 Dalton were detected from the supernatant of samples. In addition to these 14 bands two other bands (38020 and 34670 Da) were observed in the pellet of samples. In spite of different morphology of colonies there were no differences between either supernatant to each other or pellet of five *T. verrucosum* isolates.

Key words: Dermatophyte, *Trichophyton verrucosum*, Protein extraction

INTRODUCTION

Superficial mycoses in human and domestic animals caused by dermatophytes are found worldwide. The diseases have zoonotic importance because persons can be infected by contact with animals. In dairy beef production and also in public health, dermatophytoses may be of economic importance due to costs of treatment, decreased skin value and weight [1 - 3].

Trichophyton verrucosum is a zoophilic dermatophyte and is an agent of ringworm disease in human and domestic animals like camel, cow and cattle [4]. Direct contact with this fungus causes of infection of nail, skin and hair in human. The infection is usually with high inflammation such as in tinea mannum bullosa. *T. verrucosum* also makes economical lose in domestic animals [5]. It is transmitted to human through direct contact with contaminated cattle or its products in infected patients with inflammatory lesion in head, face etc. [6].

Since the proteins of fungi are used for immunization of animals thus it has generated considerable interest for determination of protein pattern and effective prophylactic programs against

dermatophytosis due to *T. verrucosum* infections in animals [7 - 10].

EXPERIMENTAL

Clinical isolates of *T. verrucosum* were obtained from veterinary faculty of Tehran University. They were identified according to conventional morphological criteria.

Extract preparation

Isolates were cultured on Sabouraud's dextrose agar and blood agar and incubated at 30 °C and 37 °C. They were transferred to 500 ml of Sabouraud's broth and incubated for 40 days at 30 °C. Mycelia were harvested by filtration and washed with PBS twice. The extraction fluid comprised borax, boric acid, EDTA and 500 ml distilled water. Freeze-thawing method and grinder instrument were used for grinding of colonies. The extracts were separated from the debris by centrifugation at 4000g, followed by 2000g for 45 min at 4 °C. Then supernatant and pellet were isolated. Protein assay was done by using Bradford method and then the extracts were concentrated by freeze drying [11 - 12].

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Electrophoretic Technique

Electrophoretic separation of proteins was carried out in resolving gel 13% and with discontinuous buffer system. Reference standards in the molecular weight range 12300-78000 da were included on each gel to facilitate comparison of the bands. Gel was stained in coomassie blue G250. Protein patterns were scanned with Helena densitometer and 590 nm filter [13 - 15]

RESULTS

The morphology of colonies were different completely but there were similarities in all protein patterns obtained from either supernatant, or pellet of isolates separately. Although the colony forms of isolates were different but no differences were seen between them (Fig 1).

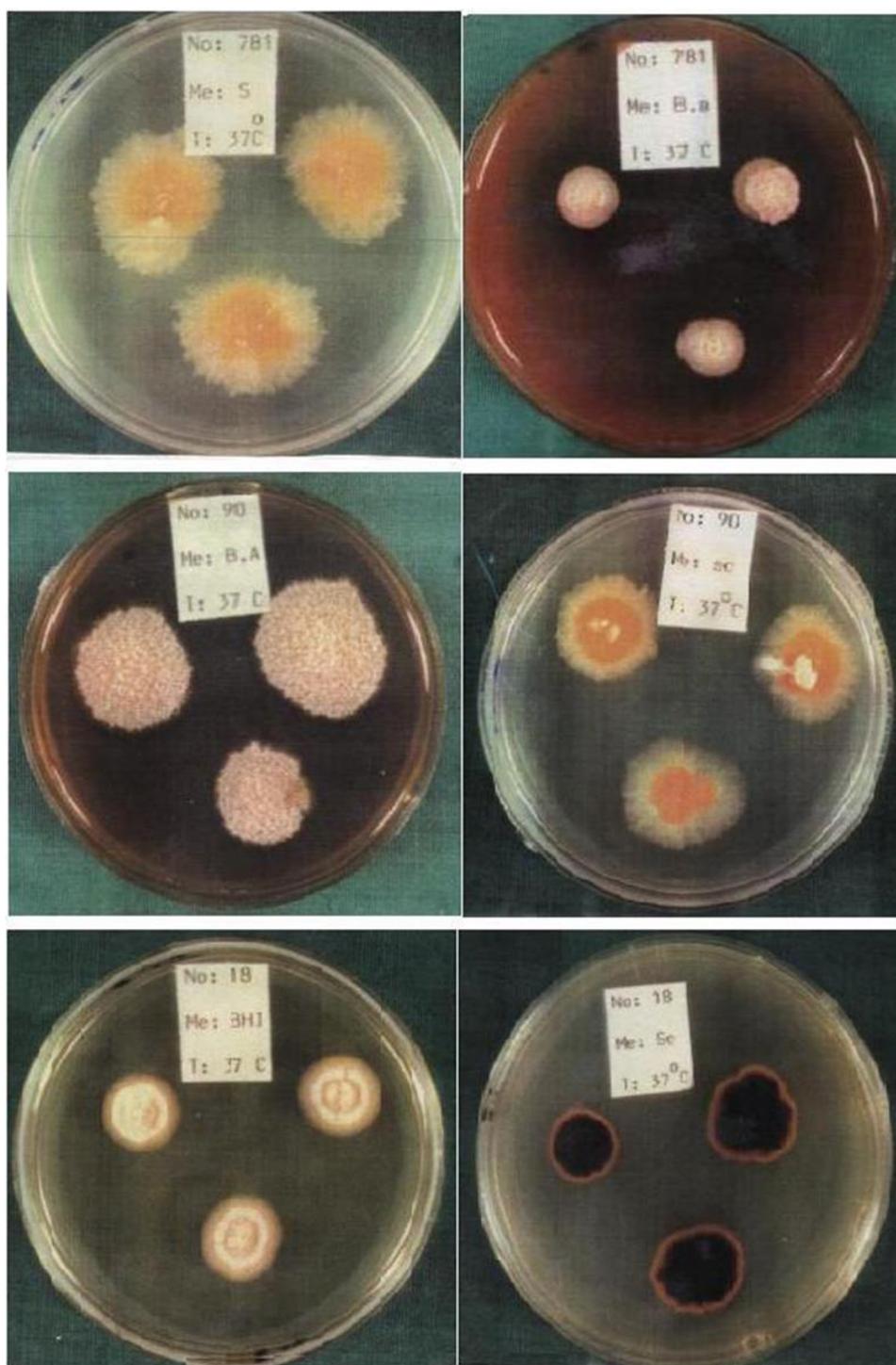


Fig. 1. The colonies of *Trichophyton verrucosum* at different media after 4 weeks

There were differences between protein patterns of supernatant and pellet in two bands. 14 protein bands with different molecular weights were observed in supernatant. In pellet of samples, 16 protein bands were detected which were the same as supernatant bands (Fig 2) and the molecular weight of two remainders were 38020 and 34670 Da (Table 1). The gel was scanned and the density of proteins were determined (Fig 3), protein standard curve was shown in Fig 4.

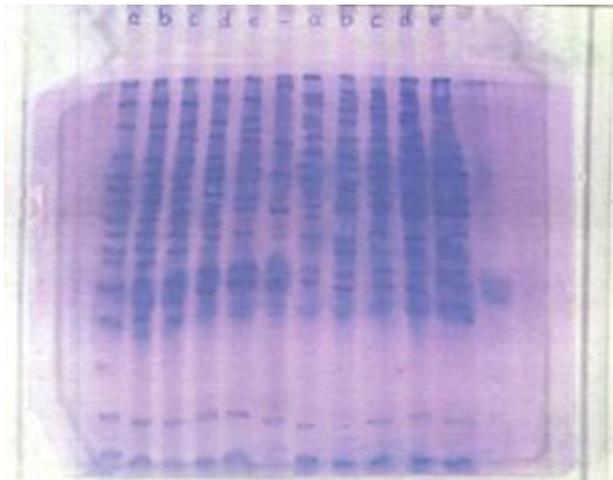


Fig. 2. Left to right a, b, c, d, e respectively: Supernatant of samples, standard, Pellet of samples

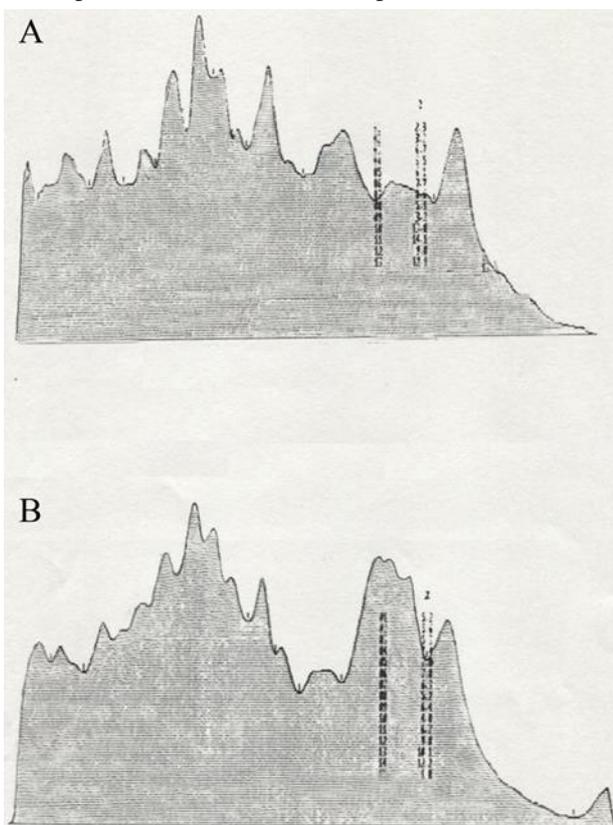


Fig. 3. Scanning of **A:** supernatant SDS-PAGE; **B:** pellet SDS-PAGE

Table 1. The molecular weights of proteins in supernatant and pellet

Mol Weights	Sample No									
	1		2		3		4		5	
	S	P	S	P	S	P	S	P	S	P
92250	+	+	+	+	+	+	+	+	+	+
81280	+	+	+	+	+	+	+	+	+	+
74990	+	+	+	+	+	+	+	+	+	+
72440	+	+	+	+	+	+	+	+	+	+
70790	+	+	+	+	+	+	+	+	+	+
63100	+	+	+	+	+	+	+	+	+	+
60000	+	+	+	+	+	+	+	+	+	+
56890	+	+	+	+	+	+	+	+	+	+
51880	+	+	+	+	+	+	+	+	+	+
48420	+	+	+	+	+	+	+	+	+	+
44150	+	+	+	+	+	+	+	+	+	+
41680	+	+	+	+	+	+	+	+	+	+
38020	-	+	-	+	-	+	-	+	-	+
34670	-	+	-	+	-	+	-	+	-	+
29150	+	+	+	+	+	+	+	+	+	+
12730	+	+	+	+	+	+	+	+	+	+

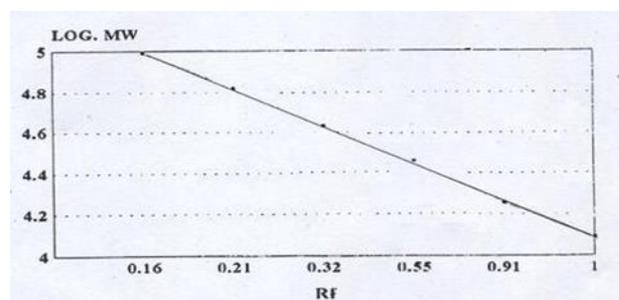


Fig. 4. Standard curve

DISCUSSION

Trichophyton verrucosum is an important zoophilic dermatophyte that causes acute dermatophytosis in human and animals [16].

This investigation was based on differences of morphology and virulence of isolates that were probably related to their proteins, but SDS-PAGE of samples showed that there were similarities in protein patterns of *T. verrucosum* isolates. On the basis of Tucker and Noble detection there was great similarity in all protein patterns of clinical isolates and no consistent differences were seen. Moreover, no differences were observed between the typical and dysgenic form of *Microsporum canis* [12]. The presence of bands 63100, 70700 and 92250 da in *T. verrucosum* and absence of them in *Microsporum canis* can probably distinguish it from other dermatophytes and specific proteins of *T. verrucosum* can be determined. On the basis of G. Grzywnowicz et al reports, *T. verrucosum* secretes enzymes into the growth medium and this strong enzymatic activity of the extracellular type of proteases is largely responsible for the relatively quick and extensive pathogenic changes. The other characterization of proteolytic enzymes of *T. gallinae* and *T. verrucosum* showed that their

properties were in many respects similar to the enzymatic activities of other dermatophytes. Therefore it would appear that like other dermatophytes a complex of several proteolytic enzymes exist [17]. Therefore the traces of protein patterns obtained were not as clear as those commonly obtained for bacteria and this may be due to the powerful proteases possessed by these fungi which were not entirely suppressed by the anti proteolytic cocktail and low temperatures employed [12].

It is concluded that different morphology and pathogenesis of *T. verrucosum* isolates may be related to enzymatic activity. Also existence of pigment may cause extensive pathogenic changes, for example *Vangiella dermatidis* and *Cryptococcus neoformance*. *Cryptococcus neoformance* without melanin shows slight pathogenesis in mouse [18]. Anyhow this is the first study about differentiation of *T. verrucosum* proteins in Iran and is introduced as protein pattern of this fungus.

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ИЗСЛЕДВАНЕ НА ПРОТЕИНИ ОТ *TRICHOPHYTON VERRUCOSUM* ЧРЕЗ ЕЛЕКТРОФОРЕЗА В НАТРИЕВ ДОДЕЦИЛ СУЛФАТ - ПОЛИАКРИЛАМИДЕН ГЕЛ (SDS-PAGE)

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Trichophyton verrucosum е един от най-честите дерматофити, който паразитира в кератинизирани тъкани на хора и животни. Това проучване сравнява протеините на пет разновидности на *Trichophyton verrucosum*, които са получени от хора и животни. Най-напред, те се култивират на специфични среди по-отделно и се инкубират при 37 °C в продължение на един месец. За получаване на протеинови екстракти, колонииите от гъбички се прехвърлят в течна среда Sabouraud и количеството на протеини се определя по метода на Bradford след растежа на гъбичките. Проведена бе SDS – PAGE електрофореза с разделящ 13% полиакриламиден гел и периодична буферна система за анализ за протеин . Кумазиново синьо G250 се използва за оцветяване на гела и се появяват различни ивици . Гелът се сканира с денситометър Helena за протеинови модели. Четиринадесет протеинови ивици с различни молекулни тегла между 12730-92250 Dalton бяха открити в супернатантата на пробите. В допълнение към тези 14 ивици се наблюдават две ивици (38 020 и 34 670 Da) в утайката на пробите. Въпреки различната морфология на колонииите, не са установени разлики между двете супернатанти както една от друга, така и за пелети от петте *T. verrucosum* изолати.