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Studies on Fungi Isolated from Dermatomycoses Patients in Egypt

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Fifty cases of dermatomycoses were recorded from adult male and female at Qena Gvernorates.These included tinea capitis (62% of total cases), tinea corporis (20%), tinea versicolor (12%) and tinea unguium (6%). Males are more susceptible to all cases of tinea than females. Thirty-one species and 2 varieties belonging to 16 genera were recovered from several infection sites. These were identified as *Aphanoascus fulvescens*, *A. terreus*, *Arthroderma fulva*, *A. obtusa*, *Trichophyton rubrum* and *T. soudanense*. Several saprophytes were also found. These were : *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cochliobolus lunatus*, *Mycosphaerella tassiana*, *Penicillium chrysogenum* and *P. citrinum*. Twenty-one isolates were able to hydrolyze gelatin with variable capabilities. *T. rubrum* was the most active protease producer. The maximum production of protease was obtained at 8 days of incubation at 30°C in Sabouraud's basal medium with maltose as a carbon source and pepton as a nitrogen source. The optinal pH for the maximum production of protease was pH 6.

KEYWORDS: Dematomycoses, Protease

Dermatophytes and other pathogenic fungi have been isolated from keratinized part of body in animals,birds and human (Gugnani *et al.*, 1975; Hubalek and Hornick, 1977; Abdel-Hafez, 1987; Ali-Shtayeh *et al.*, 1988a, b; Nichoils and Midgley, 1989; Lee *et al.*, 1990; Abdel-Hafez *et al.*, 1995; and others).

It was reported that protease had an important role in the pathogenicity of a number of microorganisms (Meevootison and Niederpruem, 1979). The ability of dermatophytes and other moulds to produce this enzyme was investigated by many researchers (Drucker, 1972; Cohen, 1973; Takiuchi *et al.*, 1982; Sanyal *et al.*, 1985; Abdel-Hafez *et al.*, 1995 and other). The optimum conditions for protease activity were studied by others (Mohamed and Turner, 1983; Afzal and Chadhary, 1991; Abdel-Hafez *et al.*, 1995).

The present investigation is aimed to study the prevalence of dermatophytic diseases and associated fungi in adult male and female in Qena Governorates (Egypt) and their capability of protease production.

Materials and Methods

Collection of specimens. Fifty specimens were collected from various parts of patient bodies (adult male and female) from march to july in 2000 at dermatology hospital in Qena Governorates. The patients were clinically diagnosed as tinea capitis, tinea corporis (tinea circinata), tinea versicolor and tinea unguium (onychomycosis). Skin scraping was collected using sterile scalpel or glass slides and was placed in sterile labelled petridishes.

Examination and culturing of dermatophytic specimens.

1-Microscopic examination: Specimens were mounted in 20% potassium hydroxide on a glass slide and covered with a slip. The preparation was warmed gently and left for 20 min before examination (Rhode and Hartmann, 1980). One drop of lactophenol cotton blue was then added and the specimens were examined using a light microscope (in positive cases the fungal hyphae, arthrospores or budding yeast cells were observed).

2-Isolation and identification of fungi: When microscopic examination was positive, the dermatophytic specimen was deposited on the surface of Sabourauds dextrose agar medium. Plates were incubated at 25°C for 2~3 weeks. The growing fungi were isolated by baiting techinque. The identification was carried out on the basis of macro-and microscopic characteristics (Carmichael, 1962; Raper and Fennell, 1965; Frey *et al.*, 1979; Domsch *et al.*, 1980; Pitt, 1985).

Proteolytic activity.

1-Screening of fungal isolates for protease production: Twenty-one isolates recovered from tinea capitis (14 isolates), tinea corporis (5) and tinea unguium (onychomycosis) (2) were screened for their abilities to produce protease. Using a sterile cork borer (9 mm diameter), the agar block inoculum was prepared. Also, holes were punched into the gelatin peptone agar. Three plates were used for each organism. The inoculum of each culture was put in the hole of gelatin peptone agar medium. Plates were incubated for 8 dayes at 25°C.

Plates were flooded with mercuric chloride solution ($HgCl_2$, 15 g; conc. HCl, 20 m*l*; dist. water, 100 m). Plates were examined for the appearance of uncolored zone, indicating protease activity. The average diameter of clear

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Table 1. Distribution of dermatophytic diseases according to sex, number and percentage of positive cases shown by direct microscopic examination and protease production

Clinical diagnosis	Number of cases	%	Sex				**	
			Male		Female		+ve cases	% of +ve cases
			No.	%	No.	%		
Tinea capitis	31	62	20	64.5	11	35.5	28	90.3
Tinea corporis	10	20	7	70	3	30	7	70
Tinea versicolor	6	12	5	83.3	1	16.7	4	66.7
Tinea unguium	3	6	2	66.7	1	33.3	3	100
Total number of cases	50	100	34	68	16	32	42	84

**Direct microscopic examination.

zones (in mm) for each isolate was measured.

2-Factors affecting protease production: The effect of physiological and nutritional factors on protease production by *Trichophyton rubrum* were studied.

a-Effect of temperature and time course: Three flasks containing 30 m*l* of Sabouraud liquid medium (pH 6.8), were incubated at 20, 25, 30 and 40°C. Flasks were removed at 2, 4, 6, 8, 10, 12 and 14 days and the mycelia were harvested by filtration using Whatmann filter paper. Filtrates from triplicate samples were combined and the combined filtrate was assayed for protease activity.

b-Effect of pH values: The test organism was cultured in the same medium which was initially adjusted to different pH values ranging from 2 to 12. Adjustment of pH was made by 0.1 N HCl or NaOH. After incubation at 30°C for 8 days, cultures were filtered and tested for enzyme activity.

c-Effect of different carbon sources: The culture medium was supplemented with 1% of each of the following carbon sources: cellulose, glucose, maltose, sucrose, wheat bran and wheat straw. Cultures were incubated at 30°C for 8 days. Filtrate was assayed for protease activity.

d-Effect of various nitrogen sources: $NaNO_3$, KNO_3, NH_4NO_3, casein, gelatin, peptone, urea and yeast extract were used as a sole nitrogen source in the culture medium. Cultures containing peptone were used as a control. After incubation at 30°C for 8 days, cultures were filtered and assayed for enzyme activity.

Assay for protease activity. Protease activity was determined according to the method described by Rick (1963). This method is based on hydrolytic action. The release of soluble tyrosine was estimated by Folin reagent. The activity was calculated from the difference between control and experimental titration value. Enzyme activity was expressed by units of L-tyrosine liberated during the reaction. The units were determined by a standard curve of L-tyrosine.

Results and Discussion

Tinea capitis. In this study, tinea capitis was the most common dermatomycoses and was emerged in 62% of total cases (Table 1). This is in a good agreement with the previous results (Chadegani *et al.*, 1987; Ekanem and Gugnani, 1987; El-Gendy, 1988; Zohdi *et al.*, 1988; Mahmoud, 1991; El-Shanawany, 1993; Abdel-Hafez *et al.*, 1995). Male was more affected by tinea capitis (64.5% of total cases) than female (35.5%) (Table 1). This result is in hormony with the finding of Zaini and Chagari (1989). *Arthroderma* (anamorph : *Nannizzia*) and *Trichophyton* were two main dermatophytes found in tinea capitis, comprising 29.03% and 22.58% of cases, respectively. Of the above two genera, two most prevalent species were *Arthroderma obtusa* (anamorph: *Nannizzia obtusa*, 22.58% of cases) and *T. soudanense* (16.13%). *Arthroderma fulva* (anamorph: *Nannizzia fulva*) and *Trichophyton rubrum* were less common (6.45% and 6.45%, respectively) (Table 2). These dermatophytes were isolated previously from cases of tinea capitis in Egypt (El-Gendy, 1988; Mahmoud, 1991; El-Shanawany, 1993; Abdel-Hafez *et al.*, 1995), as well as in other parts of the world (Al-Sogair *et al.*, 1989; El-Benhawi *et al.*, 1991). Several saprophytes were associated with cases of tinea capitis. These were *Aphanoascus fulvescens*, *A. terreus*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cochliobolus luntus*, *Mycosphaerella tassiana*, *Penicillium chrysogenum* and *P. funiculosum* (Table 2).

Tinea corporis. Tinea corporis (tinea circinata) ranked second in incidence and distribution among patient of dermatomycoses and was recovered from 20% of total cases (Table 1). This is almost in harmony with the results of Chadegani *et al.* (1987), Ekanem and Gugnani (1987), El-Gendy (1988) and Abdel-Hafez *et al.* (1995). Results revealed that male (70% of total cases) is more susceptible to this disease than female (30%). This is almost in accordance with the previous results from Egypt (El-Shanawany, 1993; Abdel-Hafez *et al.*, 1995) as well as from Libya (Elghoul *et al.*, 1989). *Arthroderma obtusa* and *T. rubrum* were two main causative species of tinea corporis and were recovered from 50% and 20% of cases, respectively (Table 2). The above dermatophytes were encoun-

tered previously in several cases of tinea corporis from patients in some Egyptian Governorates (Zohdi *et al.*, 1988; Mahmoud, 1991; El-Shanawany, 1993 and Abdel-Hafez *et al.*, 1995) as well as from many cases in other parts of the world (Shekelakov *et al.*, 1984; El Ghoul *et al.*, 1989; Katoh *et al.*, 1991 and others). *A. flavus*, *A. fumigatus* and *M. tassiana* were rarely recovered in cases of tinea corporis. Abdel-Hafez *et al.* (1995) found that

Table 2. Incidence (I) and percentage incidence (I) of dermatophytes, related fungi and other fungi associated with the different mycotic diseases

Clinical diafnosis	Tinea capitis		Tinea corporis		Tinea unguium		Tinea versicolor		Total cases	
Genera and species	I	I %	I	I %	I	I %	I	I %	I	I %
1-Dematophytes and related fungi										
Aphanoascus	3	10							3	6
A. fulvescens	1	3							1	2
A. terreus	2	7							2	4
Arthroderma	9	29	5	50			1	17	15	30
A. fulva	2	6							2	4
A.. obtusa	7	23	5	50			1	17	13	26
Trichophyton	7	23	2	20	1	33			10	20
T. rubrum	2	7	2	20	–				4	8
T. soudanense	5	16			1	33			6	12
II-Other fungi										
Aspergillus	8	26	2	20					10	20
A. flavus	4	13	1	10					5	10
A. fumigatus	2	7	1	10					3	6
A. niger	1	3							1	2
A. terreus	1	3							1	2
Cochliobolus lunatus	1	3			2	67	2	33	5	10
Mycosphaerella tassiana	1	3	1	10			3	50	5	10
Penicillium	2	6							2	4
P. chrysogenum	1	3							1	2
P. funiculosum	1	3							1	2
Total isolates	31	100	10	100	3	100	6	100	50	100
Number of genera	7	–	4	–	2	–	3	–	7	–
Number of species	14	–	5	–	2	–	3	–	14	–

Table 3. Proteolytic activities (diameter in mm.of clear zones hydrolyzed) and degree of proteolysis of fungal isolates recovered from ringworm

Organisms	Tinea capitis		Tinea corporis		Tinea unguium		Tinea versivolor	
I-Dermatophytes and related fungi	M + SD	PA	M + SD	PA	M + SD	PA	M + SD	PA
Aphanoascus								
A. fulvescens	19 + 0.82	W	–	–	–	–	–	
A. terreus	24 + 0.82	W	–	–	–	–	–	
Arthroderma								
A. fulva	35 + 0.82	M	–	–	–	–	–	
A.. obtusa	32 + 0.82	M	37 + 0.82	M	–	–	27 + 0.82	W
Trichophyton								
T. rubrum	-ve		54 + 0.82	H	–	–	–	
T. soudanense	47 + 0.82	M	–		19 + 0.82	W	–	
II-Other fungi								
Aspergillus								
A. flavus	58 + 0.82	H	27 + 0.82	W	–	–	–	
A. fumigatus	22 + 0.82	W	18 + 0.82	W	–	–	–	
A. niger	33 + 0.82	M	–	–	–	–	–	
A. terreus	28 + 0.82	W	–	–	–	–	–	
Cochliobolus lunatus	-ve	-ve	–	–	-ve	-ve	-ve	-ve
Mycosphaerella tassiana	-ve	-ve	-ve	-ve	–	–	-ve	-ve
Penicillium								
P. chrysogenum	44 + 0.82	M	–	–	–	–	–	–
P. funiculosum	20 + 0.82	W	–	–	–	–	–	–

M = mean; SD = standard deviation; PA = degree of proteolysis activity : H = high proteolysis, 50~70; M = moderate proteolysis, 30~49; W = weak proteolysis, less than 30 mm; -ve = nonproteolytic isolates; – = isolates not appear.

A. flavus and *A. fumigatus* were recovered with low incidences in cases of tinea corporis.

Tinea versicolor. Tinea versicolor occurred in 6 cases out of 50 (12%) (Table 1). This is almost in agreement with the previous results (El-Gendy, 1988; Imwidthaya *et al.*, 1989; Mahmoud, 1991; El-Shanawany, 1993; Abdel-Hafez *et al.*, 1995). Male was more susceptible than female (83.3% and 16.7% of cases, respectively). Similar findings were recorded in patients (El-Shanawany, 1993; Abdel-Hafez *et al.*, 1995). *Malassezia furfur*, the causative fungus of tinea versiolor, was detected by direct microscopic examination and was recovered in 66.7% of cases of tinea versicolor (Table 1).

Tinea unguium (onychomycosis). Three cases of tinea unguium were found (6% of total cases) (Table 1). Abdel-Hafez *et al.* (1995) found that this type of infection of the skin was least common (4.3% of total cases). In Egypt, onychomycosis was infrequent among patients of dermatophytic disease (El-Gendy, 1988; Mahmoud, 1991; El-Shanawany, 1993; Abdel-Hafez *et al.*, 1995). In present study, one dermatophytic fungus, *T. soudanense* was recovered from 33.3% of total cases of tinea unguium. *C. lunatus* emerged in 66.7% of cases of tinea unguium. It was reported that some onychomycosis were caused by saprophytes such as members of *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Scopulariopsis* and some unidentified dermatiaceous species (Velez and Diaz, 1985; Wadhawani and Srvastaval, 1985). On the other hand, several of the above fungi were frequently recovered from skin (Abdel-Hafez *et al.*, 1995).

Proteolytic activity of dermatophytes and some other moulds. Isolates were screened for their ability to produce protease. Most of isolates had the ability to produce protease with variable degree of activity (Table 3). High proteolytic activity was exhibited by *A. flavus* and *T. rubrum*. Isolates of *A. niger*, *A. fulva*, *A. obtusa*, *T. soudanense* and *P. chrysogenum* showed moderate pro-

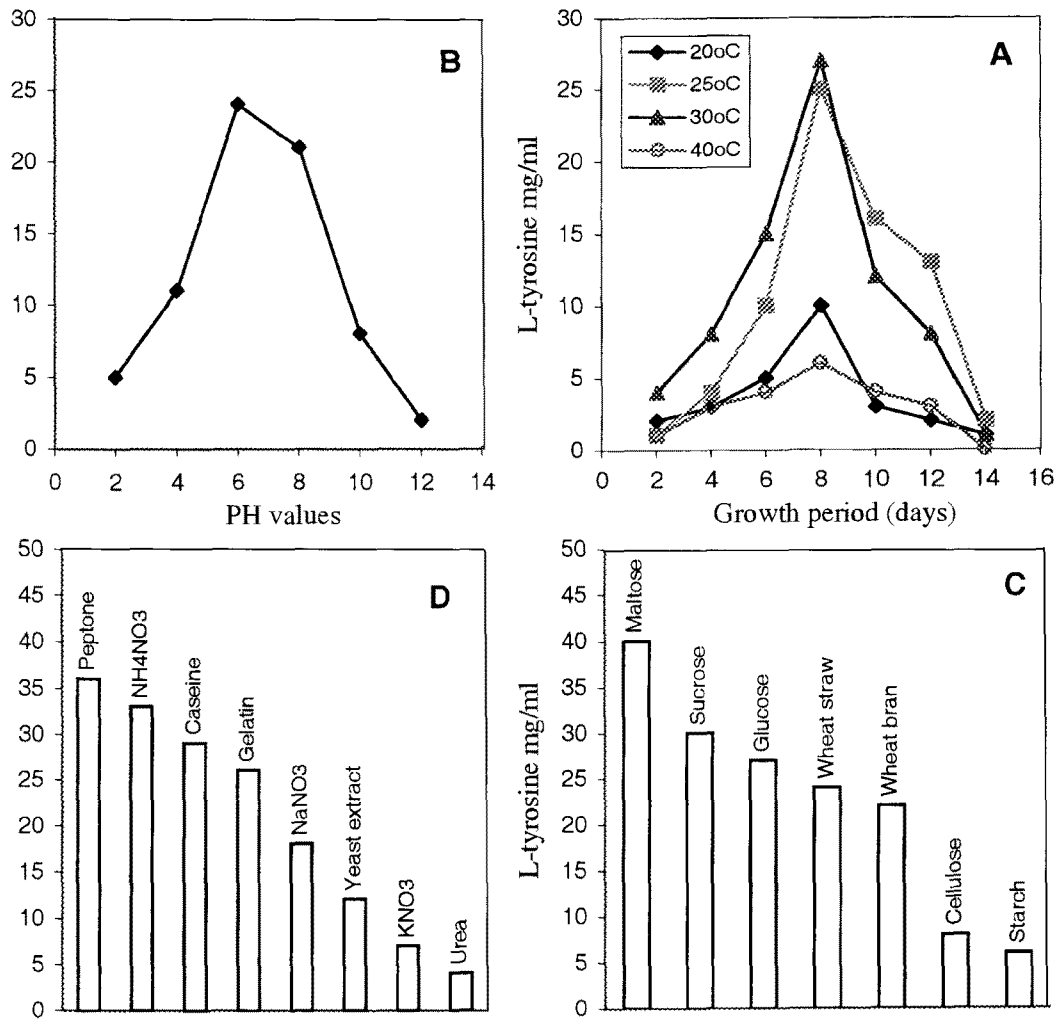

Fig. 1. Effect of time course and temperature (A), pH values, (B), different carbon (C) and nitrogen sources (D) on production of protease by *Trichophyton rubrum*.

teolytic activity. The remaining isolates were of weak or non-proteolytic activity. Abdel-Hafez *et al.* (1995) found that some isolates of *Aspergillus*, *Nannizzia*, *Penicillium*, *Scopulariopsis* and *Trichophyton* showed good protease activity.

T. rubrum was chosen to study the effects of different physiological and nutritional factors on protease production, since it was the most active dermatophytic producer (Table 3). The maximum protease production by *T. rubrum* was observed after 8 days of incubation at 30°C (Fig. 1). Abdel-Hafez *et al.* (1995) found that the protease production by *T. soudanense* was maximum after 8 days of incubation at 30°C.

The optimal pH for protease production by *T. rubrum* was recorded within a pH 6 (Fig. 1). Groninger and Eklund (1966), Lenney and Dalbec (1967) and Olutiola and Nwaogwugwu (1982) found that proteases from *Trichosporon* sp., *Saccharomyces cerevisia* and *Aspergillus aculeatus* were highly active at pH 7. In addition, Abdel-Hafez *et al.* (1995) found that the maximum protease production by *T. soudanense* was within the range of pH 6-8.

Maltose was the best carbon source for proteolytic activity of *T. rubrum* (Fig. 1). Maximum protease production was obtained by the incorporation of sucrose as carbon source in the growth medium of *A. aculeatus* (Olutiola and Nwaogwugwu, 1982), *A. niger* (Chopra and Mehta, 1985), *P. chrysogenum* (Mahmoud, 1988). Abdel-Hafez *et al.* (1995) found that maltose followed by sucrose were the the best carbon sources for proteolytic activity by *T. soudanense.*

Peptone followed by ammonium nitrate and casein were the best nitrogen sources for protease production by *T. rubrum* (Fig. 1). Abdel-Hafez *et al.* (1995) also found that casein followed by gelatin and ammonium nitrate were the best nitrogen sources for protease production by *T. soudanense.*

In conclusion, dermatomycoses recorded in this investigation were tinea capitis,tinea corporis, tinea versicolor and tinea unguium. Males are more susceptible to tinea than females. Fungal isolates of dermatophytes and other moulds were able to produce protease enzyme with variable capabilities. *T. rubrum* was the most active protease producer and the maximum production were obtained 8 days after incubation at 30°C with the incorporation of maltose as a carbon source and pepton as a nitrogen source in Sabourauds basal medium initially adjusted to pH 6.

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