Detection of Some Virulence Factors in Trichophyton. Spp Isolated from Different Cutaneous Infections

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Abstract
This study included the collection of 71 samples from Al-Sharqat General Hospital to diagnose dermatophytes. The results showed that the number of infected samples was 78.89%, while the uninfected samples were 21.11%. The results of the virulence factors of dermatophytes showed that T.interdigitale isolates produce 45.45% protease, Phosphlipase 36.36% and Ureas 27.27%, while Hemolysins 18.18 %, T. mentagrophytes isolates produce 71.42% protease, Phosphlipase 57% and Ureas 28.7%. and 42.85 Hemolysins%, T. quinckeanum isolates produce 50.0% protease, Phosphlipase 28.57%, Ureas, Hemolysins and 50.0%, Phosphlipase produtiction was 0%, T. rubrum isolates produce 71.42% protease, Phosphlipins 66.66 % And Ureas 33.33% and Phosphlipase 66.66 %.
Keywords: T. interdigital ،T. mentagrophytes، T. quinckeanum ،T. rubrum.

1. Introduction

The skin is the first line of defense for the human body and despite its tissue composition and means of protection, there are factors that help in causing pathological injuries, such as exposure to burns, wounds and weak immune system for people, especially those with diabetes, HIV and cancer patients, which increases the chance of infection with microorganisms, especially Keratin-degrading organisms such as dermatophytes (Mohammed and Al-Amy, 2012), which cause dermatomycosis, and it is one of the most common types of skin diseases worldwide, a disease that affects the skin, nails and hair caused by dermatophyte (Brasch and Glaser, 2019) and some non-dermal fungi non-Dermatophyte fungi In recent years, there has been a gradual increase in the spread of infection and it affected 20-25% of the total world population, which was represented by Dermatomycosis, Candidiasis and Pityriasis versicolar (Khurana, 2019; Pierad, 2016).

Dermatophytes produce a variety of virulent enzymes such as keratinase, proteas, phospholipase, lipase and elastase, which are produced according to the region of presence of the fungus and have an important role in the pathogenesis of the host tissues (Chinnapun, 2015). Many sequences of sequencing have been completed. Genes of dermatophytes and these sequences are used to identify virulence genes by comparing them with virulence genes of other pathogenic fungi that lead to the same pathogenic mechanism (Achterman and white, 2012). At first, it is necessary to identify the gene and determine its role in pathogenesis. One of the most important genes of dermatophytes is Which contribute to the virulence of the fungus and increase its pathogenicity, including the protease enzyme gene identified as a virulence factor such as SUB, the metalloprotease (MEP) gene and the LAP gene, which have a role in protein and keratin digestion. Components of fungal cell walls such as lignin, glucans, and glycopeptides the type of antigen stimulates a different immune response (Wagner and Sohule, 1995).

Studies have indicated that dermatophytes possess several types of virulence factors, which are major factors in the invasion of the stratum corneum, so it is necessary to identify the strategy that dermatophytes develop to escape or inhibit the host's defenses (Chinpun, 2015). Studies confirmed that the identification of virulence factors and sequence analysis the genes responsible for it are of great importance (Wagner and Sohule, 1995). The identification of the genetic sequence of virulence factors helps to reach a more effective treatment (Bitencourt et al, 2014), due to the presence of some strains that are resistant to anti-fungals (Shams-Ghahfarakhi, 2006), hoping to advance new insights to accelerate the improvement of new strategies. For the treatment of fungal skin diseases (Martinez-Rossi et al, 2018).

2. Materials and Methods

3-1 Collection Samples

A total of 71 clinical samples were collected from people presented with dermatophytosis from the dermatological lobbies at Al-Sharqat General Hospital and some private clinics in Salah El-Din Governorate during period between 25/11/2021 and 19/5/2022. The study included collecting samples from the affected areas of the skin, hair and nails under direct supervision. From the specialist doctor and for all ages and both sexes.

Skin samples were taken by scraping, as the affected area was sterilized with 70% ethyl alcohol, and then the scales were scraped from the edge of the infection focus using a sharp sterile blade. As for the hair samples, the affected hair was taken using sterile forceps and the samples were placed

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in sterile test tubes and brought to laboratories at the College of Education for Pure Sciences / Tikrit University for the purpose of examination and cultivation.

3-2: Preparation of the culture media

All culture media were prepared according to the instructions of the producing company. All culture media were sterilized with an Autoclave, at a temperature of 121°C and a pressure of 15 pounds/inch2 for a period of 15 minutes. As for the glassware and tools that were used in the experiments, they were sterilized by an electric oven at a temperature of 180 C for a period of 120 minutes (Harley and Prescott, 1996).

3-2-1: Medium Dextrose Agar Sabourauds

This medium was prepared according to the recommendations of the supplied company (Himedia) by dissolving 65 gm of (SDA) powder in 1000 ml of distilled water, then added to the medium 0.05 gm of Chloramphenicol antibiotic and 0.5 gm of Cycloheximaide to prevent the growth of opportunistic fungi. After sterilization, pour into the diameter plastic dishes. 9 mm, this medium was used to isolate dermatophytes (Emmons et al, 1974).

3-2-2: Urea medium agar

This medium was prepared according to the manufacturer's instructions by dissolving the medium with distilled water and sterilizing it with an oxidizer, then it was left to cool, then 40% urea solution was added to it under sterile conditions. This medium was used to test the ability of fungi to produce the urea-dissolving enzyme urease (Baron et al, 1994).

3-2-3: Yeast extract agar

The medium 8 g was weighed and dissolved in 1000 ml of distilled water in a glass beaker (Emmons et al, 1974). This test was used to differentiate between the genera of dermatophytes.

3-2-4: Milkagar medium

It was prepared by dissolving 28 gm in 1000 distilled water and sterilizing the medium with oxidation and after it cooled to a temperature of 50 °C. 10% of the pre-sterilized skimmed milk was added to it for 10 minutes, shaken well to homogenize, then poured into dishes. This medium was used to test fungi on the production of the protease enzyme that degrades protein (Larone, 1995).

3-2-5: Sheep blood agar

This medium was prepared by dissolving 65 gm of powdered SDA medium in 1000 ml of distilled water and sterilized with oxidizer, then left to cool to 45 °C and 70 ml of sheep blood was added to it. et al, 1994).

3-2-6: Nutritious agar medium fortified with lecithin

This medium was prepared according to the method described by Price and his group (1982) and modified by Aubaid (1997). The medium was prepared from the following materials: 20 g of nutrient agar, 1M of NaCl, 0.05M of CaCl2, 8% of sterilized egg yolk.

The yolk was prepared as a powder as follows: I took a fresh egg, separated the yolk from the albumen by a medical syringe, put the yolk in a sterile glass container, and placed the container in the electric oven at a temperature of 40 C for one hour to dry the yolk, mixing 8 g of dry matter with 50 ml Distilled water was mixed well using a ceramic mortar and the mixture was placed in a centrifuge with a capacity of 500 rpm for a quarter of an hour. Cultivation of the dishes the dishes were incubated for four days, after which the diameter of the colony and the diameter of the sedimentation zone were measured.

3-3: Biochemical Tests

3-3-1: Detection of the ability of some fungi to secrete the Phospholipase enzyme

The plates containing the culture medium (the nutrient agar enriched with lecithin) were inoculated with the fungi to be tested by transferring part of the colony in the form of a 7 mm diameter disc using a cork borer to the middle of the dishes, then the dishes were incubated at a temperature of 26 °C for two weeks, then the plates were examined to detect Enzymatic activity, the appearance of sediments around the colonies indicates the production of the enzyme Phospholipase.

3-3-2 Protease production test

The ability of the fungal isolates to produce protease enzyme by using milk medium was tested by placing a 7 mm diameter disc from the edge of the colony of the studied fungi with seven days age on the milk medium after solidification and placed in the incubator for five days at 37°C. The appearance of transparent halos around the place of cultivation is evidence of the ability of the isolated fungi to produce protease enzyme and protein analysis, and it is a positive result (Dostal et al, 2003).

3-3-3: Urea test

This test was conducted using urea medium, as the medium was poured into slant tubes and inoculated with the fungi to be tested, one left for control. Then these tubes were incubated in an incubator at 26°C for a period of seven days. The pinkish-purple color is an indication of the secretion of the enzyme (Baron et al, 1994).

3-3-4: Hemolysis test

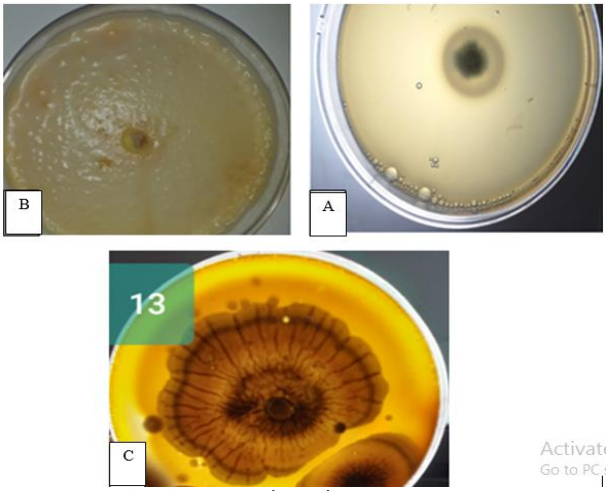
This test was used for the purpose of knowing the ability of fungi to analyze blood. A medium of sheep blood was used to detect the ability of fungi to analyze blood. A disk with a diameter of 7 mm was placed from the edge of the colony with a seven-day-old fungus planted on the SDA medium of the studied fungi at the age of seven days and placed on a medium of sheep blood agar. The dishes were incubated for 1-5 days at a temperature of 36°C, the appearance of transparent halos around the place of implantation is a positive result (Manns et al, 1994).

3. Results and Discussion

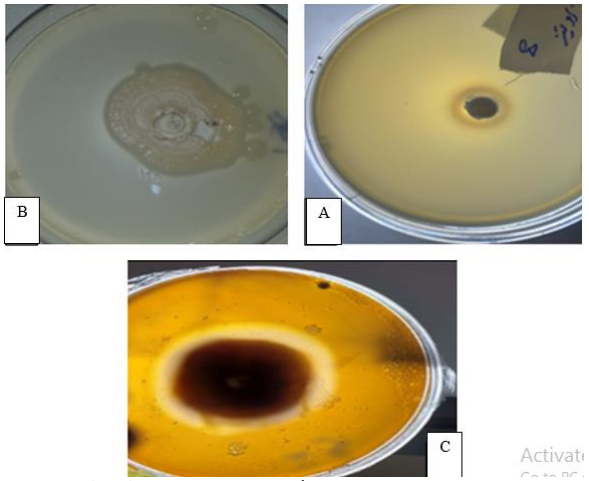
4-1 The ability of dermatophytes to produce enzymes

It was observed that the isolates of T.interdigitale

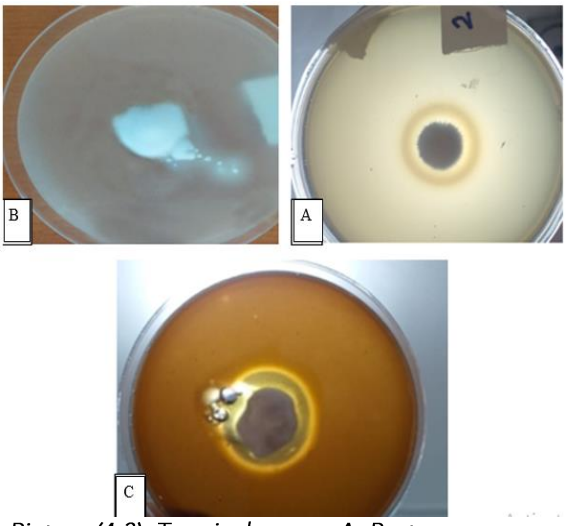
produceD protease by 45.45%, Phosphlipase 36.36% and Ureas 27.27%, while hemolysins by 18.18%, as shown in figure (4-1) and picture (4-5) Table (4-1). While isolates of T. mentagrophytes produced protease with a percentage of 71.42%, Phosphlipase 28.57%, Ureas 57.14% and Hemolysins by 42.85%, as shown in picture (4-5), table (4-1). As for T. quinckeanum isolates, they produced protease, Ureas and Hemolysins by 50.0%, while the percentage of Phosphlipase production was 0%, as shown in picture (4-5), table (4-1). It was found that T. rubrum isolates produced protease with 71.42%, Phosphlipase and Hemolysins 66.66% and Ureas 33.33%, and Phosphlipase 0% as shown in picture (4-5) Table (4-1)



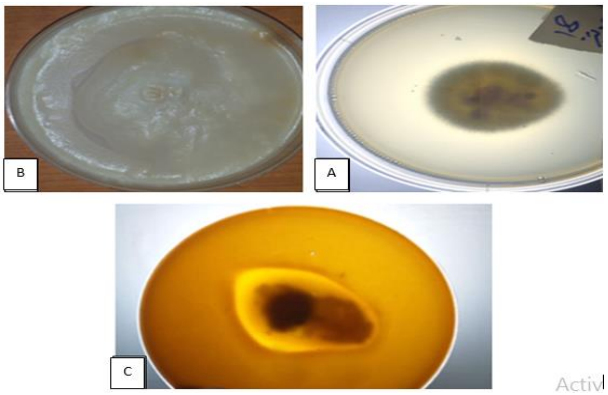
Picture (4-1): T.interdigitale A.Protease enzyme production by T.interdigitale, B. Phosphlipase enzyme production by T.interdigitale,C. Hemolysins enzyme production by T.interdigitale



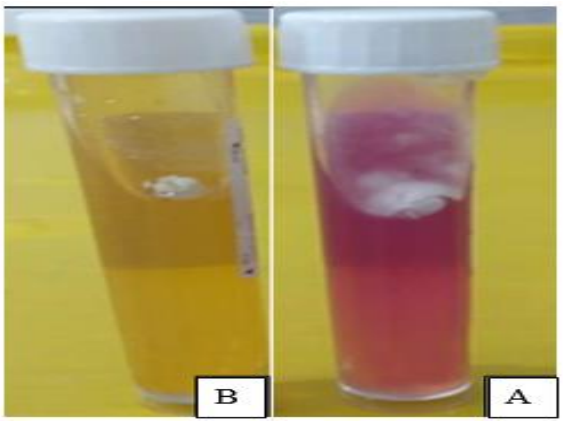
Picture (4-2): T. mentagrophytes; A. Protease enzyme production by T. mentagrophytes, B. Phosphlipase enzyme production by T. mentagrophytes.C. Hemolysins enzyme production by T. mentagrophytes



Picture (4-3): T. quinckeanum.A. Protease enzyme production by T. quinckeanum.B. No Phosphlipase enzyme production by T. quinckeanum.C. Hemolysins production by T. quinckeanum



Picture (4-4): T. rubrum.A Lack of protease enzyme production by T.rubrum.B: Phosphlipase enzyme production by T. rubrum.C. Hemolysins production by T. rubrum



Picture (4-5): Ureas enzyme.A. Ureas enzyme production by T. indotineae, T. interdigitale and T.mentagrophytes. B - Lack of production of the Ureas enzyme in T. rubrum.

Table (4-1) the ability of the studied fungi to produce enzymes

fulsolates	protease		Phosphlipase		Ureas		Hemolysins	
	Total number of produced isolates	%	no.	%	no.	%	no.	%
T. interdigitale	5	45.45	4	36.36	3	27.27	2	18.18
T. mentagrophytes	5	71.42	2	28.57	4	57.14	3	42.85
T. quinckeanum	1	50.0	0	0.0	1	50.0	1	50.0
T. rubrum	0	0.0	2	66.66	1	33.33	2	66.66
the total	11	28.94	8	21.05	9	23.68	8	21.05

These results agreed with (Aneke et al, 2021) who claimed in the results 94 (94%) and 92 (92%) strains

of M. canis produce Phosphlipase and Hemolysins, respectively. Ramos et al. (2020) found when comparing the expression of enzymes that are virulence factors for strains of M. canis isolated from dogs, cats and humans. 52 strains of M. canis recently isolated from 14 human patients, 12 dogs, 15 asymptomatic subjects and 11 asymptomatic cats were evaluated. In addition, heat tolerance was assessed by comparative analysis of fungal growth at 25 °C and 35 °C. Keratinase activity was low in 34 and moderate in 18 strains. While aspartic protease activity was low in 7, moderate in 33, and high in 12 strains. Hemolysin activity was low in 44 and average in 8 strains. All strains were classified as low catalase producers, while all strains produced urease in vitro, except for three strains and also agreed with (Rabab and his group, 2011) who isolated M. canis from tinea cruris and studied the effect of temperature on protease production. Among the factors in the growth of microorganisms and the production of enzymes such as protease, such as the temperature and pH of the culture medium and the components of the culture medium that include (sources of carbon, nitrogen and salts), the duration of incubation, as well as the ventilation and the fermentation method followed, whether it is surface or submerged fermentation (Lee et al, 1987). (

Elavarashi and his group (2017) found observed activity of virulence factors phospholipase lipase and protease from all types of dermatophytes. T. rubrum complex, T. mentagrophytes complex showed very strong positive results for phospholipase, lipase and protease activity, while others showed positive activity such as T. interdigitale produced haemolysis. A study on genome analysis of T. interdigitale/T.mentagrophytes also revealed and identified a large number of of the genes associated with virulence and pathogenicity factors such as lipase and protease (Kumar et al,2021).

The virulence factors of dermal fungi are phospholipase, lipase, protease, gelatinase and haemolytic on solid media. Phospholipase hydrolyzes convert lipids into fatty acids and other lipophilic substances. Likewise, lipase breaks down fats. Protease has proteolytic activity, breaking down proteins into polypeptides and amino acids. Gelatinase hydrolyzes gelatin into subcomponents such as polypeptides, peptides and amino acids, the gelatinase activity of dermatophytes species was analyzed for the first time in India. The medium enriched with certain substrates showed rapid growth of dermatophytes within 3-5 days of incubation indicating that they have the ability to degrade the substrate present in the skin of patients with dermatophytes for their growth. Virulence enzymes were produced by all types of dermatophytes which was similar to the previous virulence study (Muhsin et al, 1997) and thus serve as a virulence marker for dermatophytes.

In order to break down keratin, dermatophytes secrete a group of lytic enzymes that are assumed to be the main virulence factors in live tissue infection (Mercer and Stewart, 2019). associated with the secretion of enzymes to damage skin components (Graser et al, 2018) Although there is a common understanding that cutaneous keratinase is highly pathogenic, the entire process of host adaptation during infection appears to be very complex (Burmester et al, 2011).

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