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SCREENING OF THE SCALP DERMATOPHYTOSIS INFECTIONS OF BABYLON PROVINCE AND INVESTIGATION OF ITS ABILITY TO SECRETE PROTEASE

Ruwaida A Al- Aqili[1] and Ebtesam T. Jeaz[2]
Al-Qadisiyah University/College of Education/ Department of Biology.

ABSTRACT:

This research was conducted in the laboratory of the College of Education at Al Qadisiyah University and the College of Engineering Technologies at Al-Qasim Green University for the period 11/3/2022 until 4/6/2023 with the aim of investigating the fungi that cause ringworm of the head (Tinea captis). 125 samples were collected from patients visiting the dermatology consultation in Al-Hashimiya General Hospital and the private clinic of Babil Governorate / Al-Musayyib for age groups between (1-30) years ,an foor boath sexes (as the samples taken from , The samples taken from the scalp included scales and hair, under the direct supervision of the specialist doctor. The samples were diagnosed by direct microscopic examination and the remaining part of the pathological samples were cultured on SDA supported with Cycloheximide and Chloramphenicol. Through direct microscopic examination of the fungi, positive results were revealed in a rate of infection of 36% (125) samples, while positive results of laboratory culture were shown in a rate of 48.8% of the total 125 samples.

The results of phenotypic examinations of the isolated skin fungi showed that they belong to the two genera, namely Microsporum and Trichophyton. The results found that the genus Microsporum is one of the most diagnosed specimens compared to the genus Trichophyton, the least of the fungal specimens, and from which two species of Microsporum were isolated, namely Microsporum. Canis and Microsporum. gypsum, where Microsporum.canis had the highest percentage (44.44%) and the percentage of *Microsporum gypsum* (13.33%). As for the genus *Trichophyton*, four species were isolated from it: *Trichophyton mentagrophytes, Trichophyton tonsuranis, Trichophyton rubrum*, and *Trichophyton verrucosum. Trichophyton mentagrophytes* had the highest percentage (13.33%) and Trichophyton the lowest percentage. rubrum(4.44%).regard to protases secretion efficiency we found

Keywords: *Microsporum. canis* ; Tinea captis; Molecular detection; *ringworm*

Introduction

Skin diseases are one of the common health problems that have the ability to attack the keratinized tissues of humans and animals, such as hair, skin, and nails, causing superficial fungal infections of the skin known as Dermatophytosis. They include three genera: *Trichophyton, Microsporum*, and *Epidermophyton*. They may cause diseases that vary in terms of their severity, development, and location of infection (Al - Harbi *et al*., 2022). Diseases caused by skin fungi are divided according to the site of infection, including: Tinea captis, Tinea corporis, Tinea manuum, Tinea facie, Tinea pedis, and Tinea cruris. Therefore, skin fungi are the main cause of one of

the most important skin diseases, the incidence of which has increased at a rate of significant over the past years (Whiteand Achterman,2011). Tinea capitis is a common infection that affects the hair and scalp and occurs mainly in children. Tinea capitis occurs due to either anthropophilic or zoophilic causes. Geophilic dermatophytes are less common throughout the world. *Trichophyton violaceum* and *Trichophyton tonsurans* are considered the most common types of fungi.

The human-loving dermatophyte fungus is isolated most often, while the fungus *Microsporum canis* is the most widespread skin fungus (Rodriguez *et al.,* 2021). The fungus Microsporium canis has found it to be a major factor in skin infections in cats and dogs, and it is also transmitted to humans. As a result, the rate of human infection has increased in European countries, and this is due to direct contact with infected animals, and it has become the dominant factor causing ringworm (Tinea capitis) in European countries. And South America and the Middle East, and among the skin infections caused by this fungus are ringworm (Tinea corporis), ringworm (Tinea capitis), ringworm (Tinea favosa), ringworm of the chin (Tinea barbea), and mycetoma (Jańczak *et. al*., 2023). It was also noted in a study on ringworm in some countries, including Brazil, that the *M. canis* type is the spreading agent that causes ringworm, with a rate of 70.5%, while the rate of the inflammatory type Kerion, which is caused by the same type, was about 44.4% (De Moroaes et al, 2000).

It was noted that the rate of fungal infections in Iraq is that the two sexes, M. ferruginum and T. schoenleinii, are responsible for ringworm of the head in Mosul (Mahmood, 2023), and it was found that the rate of infection of ringworm of the head caused by the fungus M.canis reached 55.1% in Baghdad (Ali & Yassein, 2022).

Materials and Methods

Collection of Samples : This resesarch was conducted on ten isolates of Diagnosis of M.canis 125 samples were collected from the private clinic in Al-Musayyab District / Babil Governorate, as well as from Al-Hashimiya General Hospital for people suffering from ringworm of the head, for a period ranging from November 20/11/2022 to April 6/4/2023. The study included 67 male samples and 43 samples. Of females and of age groups ranging from one year to thirty years, by taking scales and hair from the affected area, a questionnaire form was recorded that included information about the infected person (gender, age, occupation, residence, accompanying symptoms).

I planted part of the fungal samples, represented by peels and hairs, which were not treated with potassium hydroxide solution, as they were grown on culture media (SDA), and then the antifungal Cyclohexmide was added to it to prevent the growth of saprophytic fungi, and the antibiotic Gentamycin was also added to prevent bacterial growth. (Kannan et al.,2006). Identification of fungal isolates by Colonies the of examination morphology Colonies of the microscopically examination *M.Canis* phospholipase Hair penetration test Cocking rice Urase Production test (Baron *et al*

.,1994) . A PCR test was performed to diagnose the fungus *M.canis* to confirm the phenotypic and microscopic diagnosis, as this fungus is important as a cause of tinea capitis.

Protolytic activity test: The medium was to prepare by dissolving 20 gm of agar in 900 ml of the distilled water in the beaker. Where 10 gm of skimmed powdered milk in 100 ml of the distilled water in another beaker. Then the two solutions were sterilized in an autoclave, each of them separately, and then they were mixed together after cooling to the temperature. 45°C, then add the antibiotic**,** Chloramphenicol 250 mg/L, then pour it into dishes and leave it to solidify. The medium was inoculated with a 5 mm disc of a pure colony , After that, it was incubated at temperature of 27°C on 3 days. This medium was used for the purpose of detecting the ability of fungi to degrade protein by producing the protease enzyme. The area appeared clear or transparent around the colony, and this indicates a secretion. Protease enzyme (Aaronson, 1973).

Primers used in the study : The PCR primers in the study were designed using GenBank on the NCBI global biological information website, using Primer 3 plus, and these primers were prepared by the Korean company ABM. Table (1) which is as follows:

Table -1- Primers used in the study with the sequence of the nitrogenous bases and the size of the primer

Primer		Primer sequence (5′-3′)	Amplicon
Protease	F	5- CGTACGACTGGATCGTCAAG-3	404 bp
	R	5- AGATGTCCACGATCTTGCCG-3	

The polymerase chain reaction mixture was prepared in PCR tubes, other components were added to the reaction mixture according to the company's instructions, the mixture was transferred to the Vortex device for ten seconds, and the mixture was placed in the Thermo Cycle PCR to complete the DNA replication process, This is shown in a(Tabe 2).

Table (2): The Reaction mixture and volume.

Mixture Contents	Volume (µl)
PCR PreMix(5-50 ng)	**25ul**
Forward primer (10pmol)	**3ul**
Reverse primer(10pmol)	**3ul**
DNA template	**5ul**
PCR water	**14ul**
Total volume	**50ul**

In this research, PCR terminology was used with the prefixes where the initial mutant , Initialdenaturation at 94 ° C for five minutes, one cycle Followed by 35 cycles

of denaturation at 94 ° 1 minute annealing at 55 ° C for minute, 35 cycles as well At 72 ° C for 1 min, 35 cycles and final extension at 72 ° C for 10 min, 1 cycle (Table 3).

Table (3): Reaction Conditions used in the PCR

Phase	Tm(C)	Time	No.of cycle
InitialnDenaturation	94C	5min	**1cycle**
Denaturation	94C	1 Min	
Annealing	*55C*	1min	**35 cycle**
Extension	72C	1.5min	
Final extension	72C	10min	**1 cycle**

Gel electrophoresis: According to the (Samobrook *et al.*, 1989) method for preparing agarose gel, to perform electrophoresis as a result of analyzing and reading the polymerase reaction of the protease enzyme. The gel was prepared by dissolving it at 1.5°C, then adding 5ul of TBE buffer using a microwave for five minutes, then leaving the gel at a temperature of 45-50°C, then adding 5ul of ethidium bromide dye and mixing it well. After that, it was transferred into the transfer molds containing the comb and left in it. Until it hardens for half an hour, the samples are loaded by adding 5ul to each of the PCR products, and finally the agarose is immersed in TBE solution, after which the samples are electrophoresed under a voltage of 5v for an hour and a half, and the gel is examined using ultraviolet radiation at a wavelength of 302 nm.

RESULTS and DISCUSSION

The result was after molecular investigation of the virulence enzymes, showed that the fungus was infected The *M. canis* isolate possessed the pro gene after being electrophoresed and stained with ethidium bromide dye, and examined with ultraviolet light. The DNA bands were approximately (404bp), as in Figure (1).

Dermatophytes are characterized by their ability to degrade keratin, which affects the hair and nails and, in the case of the human host, causes skin infections. Proteins are considered essential in the assimilation of creatine, and proteins similar to (SUB 1) Subtilisin 1 and (SUB 3-7) are specific to dermatophytes (Tang *et al.* ., 2023).

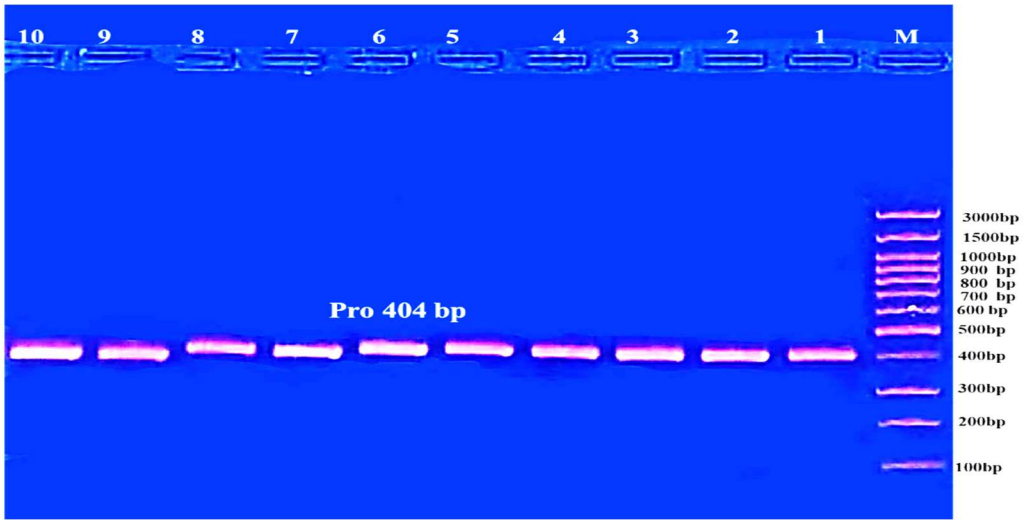

Figure (1): Protease gene amplification products for the fungus *M.canis* under study. The electrophoresis was carried out on a 1.5% agarose gel, at a voltage of 70 and a voltage of 80 amps, for one hour, with a DNA ladder maker (M = 100-3000bp).

Protease production test : Figure (2) shows the result of the test for the ability to secrete the proteolytic enzyme of the fungus *M. canis*. The test gave a positive result when the fungus was grown on skim milk medium. It was diameter of the transparent halo that formed around the fungus was 20,22 mm. This is an indication of the decomposition of the casein protein present in The medium is milk, as the activity of the enzyme is measured according to (Nakagowa, 1970), where the activity is high if the diameter of the areola is greater than 15 mm.

Filamentous fungi produce types of proteolytic enzymes. These fungi grow in different environmental conditions of temperature and pH, and their effectiveness increases when nitrogen sources are available, such as the protein casein, peptone, aspartic acid, and gelatin (Nehra et al., 2002), as protease enzymes play a major role in the disease. Fungi, as these enzymes work to decompose the host's tissues and turn them into food that it uses to support its growth and sustain its other vital processes. They also contribute to paralyzing the host's defenses and facilitate the ability of the fungus to penetrate the tissues and cause disease (Martínez-Herrera *et al.,* 2023). This result is consistent with what was reported by (Ramos *et al.,* 2020), who isolated the fungus and studied its enzymatic capacity.

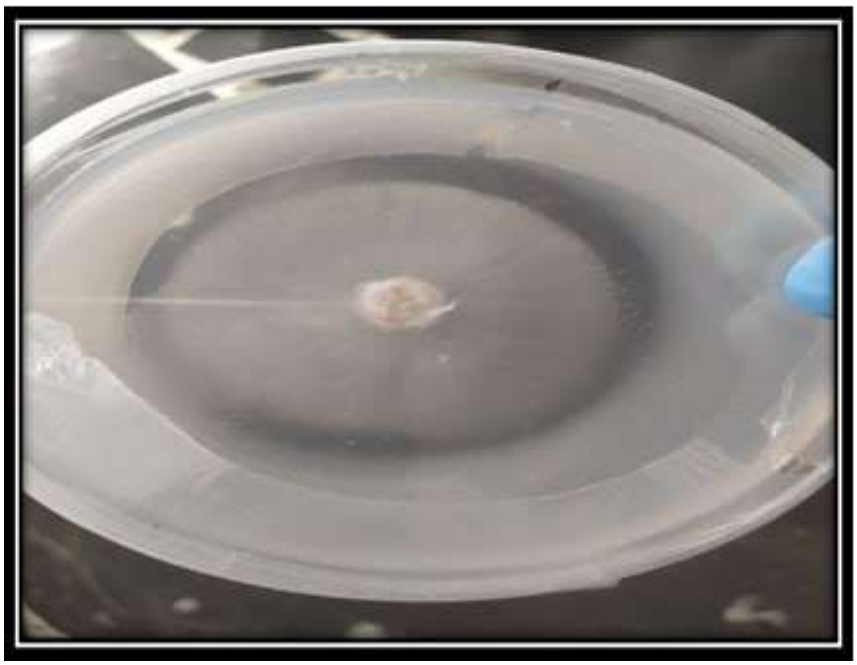

Figure (2): Test form for the ability of the fungus *M.canis* to secrete the protease enzyme

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