# Identification Of *Trichophyton rubrum* Using Polymerase Chain Reaction

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## Summary

*Trichophyton rubrum* is an anthropophilic dermatophyte that is distributed worldwide and causes common cutaneous disease such as mycosis . Although several properties of this fungus have been investigated so far , however , a few studies were carried out in the field of molecular biology of this fungus .

In the present study the application of PCR fingerprinting was performed using two primers : forward 5'TGGTCTGGCCTTGACTGACC3' and Reversed 5 ' GTAAGGATGGCTAGTTAGGGGG 3 ' for the purpose of species identification .

*Trichophyton rubrum* isolates obtained from either human patients (5 isolates) and animals ( 5 isolates ) with dermatophytosis were prospectively isolated by cultures and identified on morphological basis at Baghdad University , Department of Dermatology , College of Medicine and College of Veterinary Medicine respectively from the period September 2010 till March 2011 .*Trichophyton rubrum* isolates were subjected to DNA extraction .Conventional PCR was done with *Trichophyton rubrum* specific primers 5 ' TGGTCTGGCCTTGACTGACC 3 ' and 5 ' GTAAGGATGGCTAGTTAGGGGG 3 ' .

Six isolates were positive for DNA extraction .A single band corresponding to *Trichophyton rubrum* was obtained .

The results of current study suggest that PCR is simple , rapid and sensitive method for diagnosis of dermatophyte infections .

**Key word:** *Trichophyton rubrum* identification, PCR .

## تشخيص الفطر ترايكوفايتون روبرم بأستخدام تفاعل البلمرة المتسلسل

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## الخلاصة

تعتبر الترايكوفايتون روبرم من الفطريات المحبة للبشر متوزعة في العالم وتسبب الاصابات الفطرية الجلدية مثل المايكوسس . وبالرغم من ان هذة الصفات العديدة لهذا الفطر تم التحري عنها ولكن هنالك دراسات قليلة اجريت في حقل البايولوجي الجزيئي في هذا المجال .في هذة الدراسة تم استخدام فحص البلمرة المتسلسل بوجود نوعان من البرايمير :

الاول: ' 3 5 ' TGGTCTGGCCTTGACTGACC

والثاني : ' 3 5 ' GTAAGGATGGCTAGTTAGGGGG

لغرض التشخيص .تم الحصول على خمسة عزلات مأخوذة من الانسان وخمسة عزلات من الحيوانات حيث تم تشخيصها بواسطة الزرع الفطري في شعبة الامراض الجلدية/ كلية الطب /جامعة بغداد وكلية الطب البيطري /جامعة بغداد بالتسلسل للفترة من ايلول /2010 ولغاية أذار /2012 وقد اخضعت هذة العزلات الى استخلاص DNAمع أستخدم تفاعل البلمرة المتسلسل العادي وكانت ستة عزلات اعطت وجود DNA وتم تحديدها على شكل Band.

هذة الدراسة تقترح بأن فحص تفاعل البلمرة المتسلسل هو بسيط ,وسريع وطريقة حساسة لتشخيص أصابات الفطريات الجلدية.

**الكلمات المفتاحية**:- الذاكيوفاينون , وبرم , الشمس , تفاعل البلمرة المتسلسل .

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## Introduction

Dermatophytes are keratinophilic fungi able to infect keratinized tissues of human or animal origin , leading to infections that are mainly restricted to the corneocytes of skin , hair and nail ( 1 ) . These filamentous fungi are usually identified on the basis of clinical feature and isolation patterns together with conidial morphology , and some time with physiological characters , such as the hair perforation and urease test ( 2 ) . In some cases, morphological identification can be difficult or uncertain because there is considerable variation and pleomorphism among isolates of the same species. In the last decade , genotyping approaches have proven to be useful for solving problems of dermatophyte taxonomy as well as enhancing the reliability and speed of dermatophytosis diagnosis ( 3- 8 ) .In the present study the fungus was identified by culture characters and DNA amplified method called PCR.

## Materials and Methods

Fungal strain: Ten *T. rubrum* isolates obtained from either human patients with dermatophyte ( 5 isolates ) or animals with or without visible lesions ( 5 isolates ) were prospectively isolated by culture and identified on the basis of their morphological feature ( 1,2 ) at the University of Baghdad , College of Medicine ,Dermatological Department and College of Vet Medicine , Baghdad University respectively from September 2010 to March 2011 .

All strains were grown at 30C on Sabourauds Dextrose agar Medium with chloramphenical plus cycloheximide for 3 weeks . They were identified on the basis of microscopy and colony characteristics and were classified using the following criteria ( i ) : clinical criteria body localization associated or not with clinical signs or symptoms of dermatophytosis ( ii ) cultural aspects (texture , front and reverse colour of the colonies) and ( iii ) microscopic features subspherical or clavate microconidia , macroconidia, spiral hyphae ( 5, 8 )

DNA extraction : DNA was extracted from a small amount of mycelium grown on Sabouraud dextrose agar by a rapid preparation methods ( 9 ) . Mycelial cells were disrupted by vigorous shaking with 0.5 – mm – diameter zirconium . silica beads ( Azygen Scientific , Union city , CA) in a mechanical cell disrupter ( Mini – Beadbeater ; Biospec Products , UK ) as previously described ( 10 ) . The disrupted cell suspension was centrifuged at 8 , 000 x g for 10 min in a microcentrifuge . Fungal DNA in the supernatant was extracted by a genomic DNA extraction kit (Alpha DNA – Canada) according to the manufacturers instructions.

Polymerase chain reaction was then performed using a hot star Taq polymerase plus Master Mix kit (Promega – USA) according to the manufacturer's instructions. The following primers were used that amplify a 280 base pair fragment containing a GT – microsatellite repeat specific for strain of *Trichophyton rubrum* forward 5'TGGTCTGGCCTTGACTGACC3' and reversed5' GTAAGGATGGCTAGTTAGGGG3' (Alpha DNA – Monterial Canada).

The fungal DNA was amplified by 30 cycles in a thermocycler ( Thermomixer confort ; Eppendorf , Hamburg , Germany : 30s at 95 C denatoration ,30 s at 60 C annealing and 45s at 42 C extension . The amplification product was then checked for bands with 280 bp by gel electrophoresis in a 2% agarose gel in comparison with a negative control with DNA extracted from *Trichophyton rubrum* .

## Result

Ten strains of *Trichophyton rubrum* were subjected to DNA extraction as shown in figure (1) . This figure shows lane number ,bands of chromosomal DNA isolated from *Tichophyton rubrum.* This figure shows no plasmid band. Only six isolated of *Trichophyton rubrum* gave band. A 280 bp band corresponding to *Trichophyton rubrum* was observed in the PCR fingerprint (figure 2). The etiological agent ( *Trichophyton rubrum* ) in this study was thus identified .

In general, gross examination of the fingerprint profiles of the six *Trichophyton rubrum* isolates revealed that all isolates were generally similar .

The reasons for the genetic homogeneous of these Iraqi isolates of *Trichophyton rubrum* is not clear, but it may be due to the limited region from which isolates were obtained .

## Discussion

The isolation and purification of DNA is a key step for most protocols in molecular biology studies and all recombinant DNA techniques (11) .

Misidentification using conventional microbiological methods was one of the main reasons of that dermatophytes were among the first fungal groups studied using molecular genetic methods (12).

Polymerase chain reaction has rapidly become one of the most widely used techniques in molecular biology for its high discriminatory power and reproducibility and because it requires very little starting material and is rapid , simple means of producing relatively large number of copies of DNA molecular from minute quantities of source DNA materials (13). This result showed that the PCR method used allows identification of *Trichophyton rubrum* than the commonly used combination of KOH mount plus culture. This results was in agreements with the of other (14, 15).

The current diagnosis of dermatophytes is based on microscopic identification of spores and hyphae in clinical specimens followed by in vitro culture and morphological identification of the fungus (16). Direct microscopic examination of skin and nail materials is often sufficient for the preemptive diagnosis of a fungal infection , but it does not give specific species diagnosis .Furthermore , although rapid and cheep , this technique has a relatively low sensitivity and shows false negative results in up to 15% cases (17).

Application of culture enables specific species identification in 10 – 15 days in approximately 95% of cases. However, for some slow growing or a typical isolates need time to diagnosis is

up to 3-4 weeks. Such cases are especially cost and time consuming and require specialist skills (4). It is thus obvious that a simple, rapid and specific method for the diagnosis of dermatophyte infection is necessary. Introduction of PCR based methodology would increase specificity, simplicity, speed and on the same time be inexpensive (18).

In Conclusion , isolation and identification of dermatophytes by culture remain to be the strongest evidence in diagnosis of fungal pathogen , PCR analysis may be an alternative method to identify dermatophytes.

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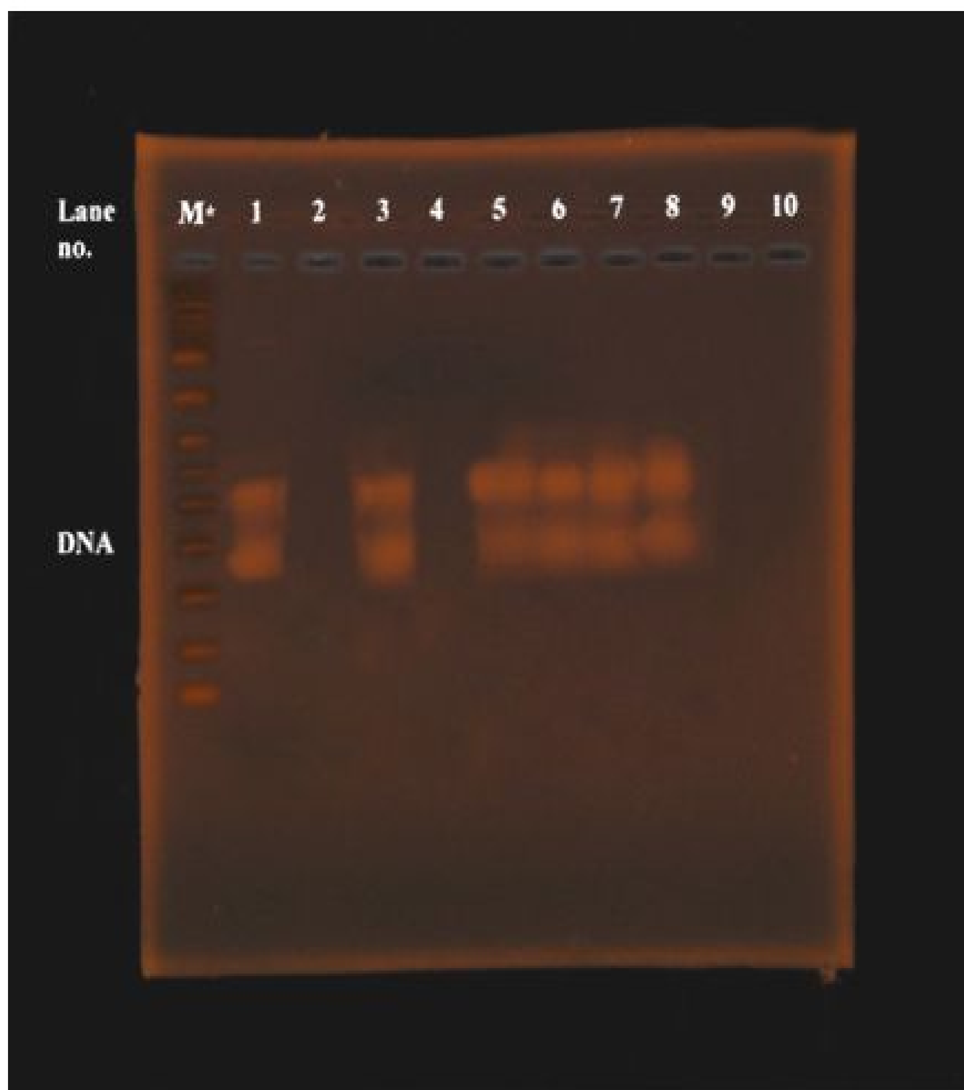


**Figure( 1 ):Gel electrophoresis of Total DNA extracted from *Trichophyton rubrum* strains ( agarose 2.0% - 100 mV - 2 hours ). M* ( Molecular weight marker )**

Note: Ten *Trichophyton rubrum* isolates from human patients (5 isolates) and animals (5 isolates) were included in DNA extraction.

(1,3.5,6,7,8)= No. of isolates , (2,4,9,10) = Negative DNA

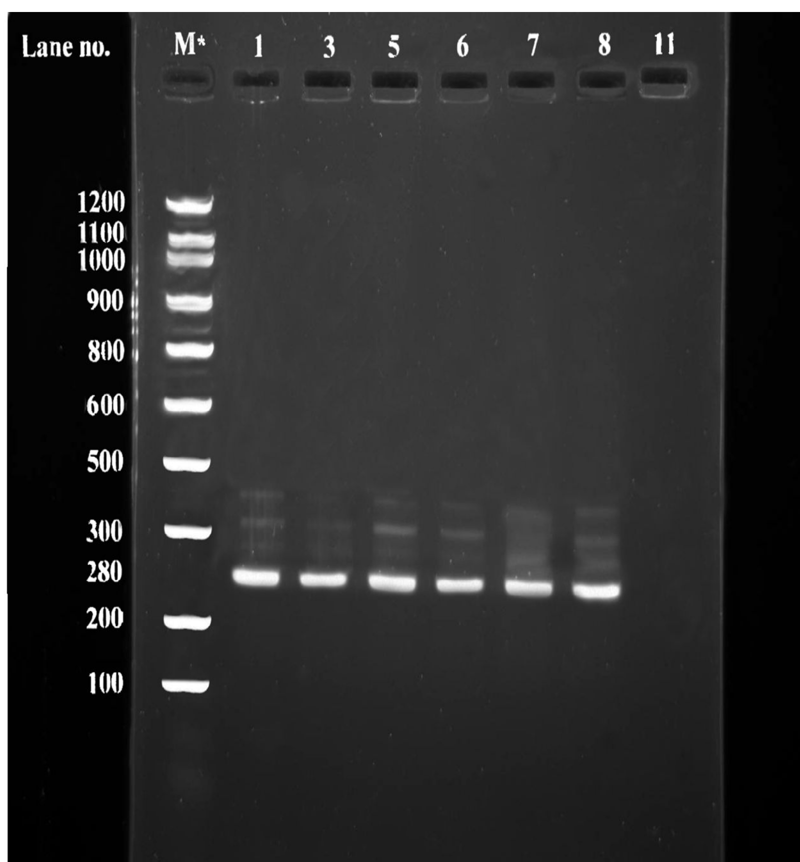


**Figure (2):*Trichophyton rubrum* specific band. Lanes(1,3,5,6,7,8) show positive bands with 280 base pairs (bp) of different strains of *Trichophyton rubrum* . Eleven = negative control (agarose gel 2% - 100 mV- 2 hours ). M* (Molecular weight marker )**

*Note: 1, 3, 5, 7 ( Band from animal isolates ) , 6 , 8 ( Band from human isolates ).