Molecular Identification of *Trichophyton* Spp. by PCR-RFLP

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الملخص

خمسة و سبعون نوعاً من الشعروية (48 الشعروية المنتاجروفايتس متغيرة منتاجروفايتس ، 17 الشعروية الانتروديقيتالي ، 1 الشعروية المنتاجروفايتس متغيرة ايرينسي ، 7 الشعروية التونسيورانس و2 الشعروية الروبرم) تم تصنيفها بواسطة تقييد طول القطعة متعدد الأشكال باستخدام إنزيم م ف1. نتج عن ذلك خمسة نماذج مختلفة إثنين الي أربعة شرائط. لم تعطي الأنواع المختلفة نفس النموذج المظهرى.

Summary

Seventy-five *Trichophyton* spp. isolates (48 *Trichophyton mentagrophytes* var *mentagrophytes*, 17 *Trichophyton interdigitale,* one *Trichophyton mentagrophytes* var *erinacei,* seven *Trichophyton tonsurans* and two *Trichophyton rubrum*) were identified using restriction fragment length polymorphism (RFLP) using *Mva*1 enzyme. Five different patterns of two to four bands were obtained. None of these different species gave the same profile pattern.

Introduction

In recent years, DNA analysis has been too refined to make genetic-based differentiation of dermatophyte species possible although such methods are not available for routine practice. Liu *et al.* (1996) used random primer 5'-ACCCGACCT G-3' for genomic DNA to differentiate *Trichophyton* spp. Mochizuki *et al.* (2003) used the restriction enzymes *Mva*I and *Hinf*I to digest amplicon DNA. They reported that *Mva1* showed two to four bands for *Trichophyton tonsurans*, *T. quinkeanum*, *Arthroderma simii* type 2, *T. verrucosum*, *T. rubrum*, *T. violaceum* and *Epidermophyton floccosum*. PCR-RFLP with digested enzyme *Hinf*I showed two to five bands for *T. mentagrophytes* var *erinacei*, *T. concentricum* and *A. benhamiae* (Mochizuki *et al.*, 2003). The present study aimed at the identification of *Trichophyton* spp. by RFLP in the Sudan.

Materials and Methods

Dermatophytes isolates

Forty-eight *Trichophyton mentagrophytes* from Sudanese camels, 17 *T. interdigitale* human isolates, one *T. mentagrophytes* var *erincei* and seven *T. tonsurans* (3 camel isolates and 4 human isolates) were used in this study, besides two *T. rubrum* from Westmead hospital (Reference isolates).

DNA Extraction

The isolates were grown in Sabouraud's Dextrose Agar at 27°C for two weeks. The mycelia were scraped with a scalpel, transferred to 1.5ml Eppendorf tube containing 700 µl deionised water, and centrifuged for 15min at 14000 rpm. The DNA was extracted as described by Meyer *et al.* (1999). To test the quality of the extracted DNA, 2µl of loading buffer was added to 2µl DNA, which was visualized as a single band in 1.4% agarose gel. The DNA concentration was estimated by measuring the optical density at 260 nm.

Amplification of ITS region

The reaction was performed in 100 µl volume containing 100 ng of genomic DNA, 10X PCR buffer (100mM Tris-HCl, pH 8.3, 500mM KCl, 15mM $Mgcl_2$, 0.01% W/V gelatine) 4mM of dATP, dCTP, dGTP and dTTP each, 100mM Mg acetate, 5U/ µl of Taq

DNA polymerase and 50ng of LR1 primers(5'-GGTTGGTTTCTTTTCCT-3') and SR6R (5'-AAGTAAAGTCGTCGT AACAAGG-3'). The sample was overlaid with light oil sterile (Sigma) and amplified in the thermocycler (Perkin Elmer); the programme was started with an initial denaturation at 97 ºC for 3 min followed by 20 cycles at 97 ºC for 35s, 50 ºC for 55s and 72 ºC for 45s. This was followed by 10 cycles at 97 ºC for 45s, 50 ºC for 55s and 72 ºC for 2 min and finally 72 ºC for 6 min. The amplicons were detected as a single band by agarose gel electrophoresis (Graser *et al.*, 1999).

RFLP-ITS analysis

The ITS amplicon was digested with restriction enzyme *Mva*1 (TAKARA, BIO, INC, Japan). The mixture contained 25µl of the ITS product, 3µl of 10X buffer (10mM Tris-Hcl, pH 8.5, 15mM $MgCl_2$, 150mM KCl, 1mM DTT, 0.01% BSA) and 0.3µl of the enzyme and incubated for 3 hrs at 37 ºC. After being electrophoresed in 3% agarose gel, it was stained with ethidium bromide and visualized under UV (Jackson *et al*., 1999).

Results

The universal fungal primers LR1 and SR6R amplified the ITS1 5.8S and ITS2 regions for all the 75 dermatophytes isolates studied showing single band (Fig. 1). The ITS1 and ITS2 regions amplified for all *Trichophyton* spp. studied was 775 bp. The use of *Mva*1 restriction enzyme on *T. mentagrophytes* var *mentagrophytes*, *T. mentagrophytes* var *interdigitale*, *T.tonsurans*, *T. mentagrophytes* var *erinacei* and *T.rubrum,* showed two to four bands. Five patterns were obtained from all *Trichophyton* spp. isolates. All the 48 *T.mentagrophytes* var *mentagrophyte*, the 17 *T.mentagrophytes* var *interdigtale*, the seven *T. tonsurans* and the two *T.rubrum* showed an identical profile each species. Results are illustrated in Fig. 2.

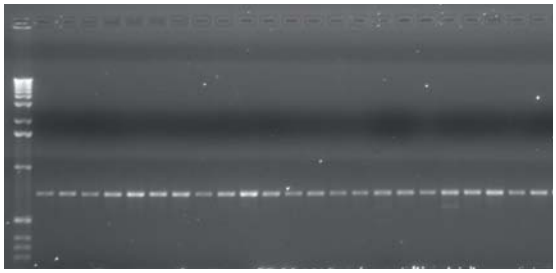

Fig. 1: Single band as result of amplified ITS region of *Trichophyton* spp.

Discussion

Accurate identification of dermatophytes is time-consuming and requires extensive familiarity with the microscopical and cultural characteristics of these taxa. In this study, we were able to distinguish between the five species of dermatophytes by producing five different patterns using the *Mva*1 enzyme in a RFLP assay.

*Mva*1 restriction patterns are consistent and highly reproducible, indicating that the ITS regions in dermatophytes are conserved.

Fig. 2: RFLP profiles of *Trichophyton* spp isolates. WM 07.322, WM 07.323, WM 07.327, WM 07.339 were *T.mentagrophytes var mentagrophytes*, WM 06.837, WM 06.871, WM 06.884, WM 06.861 were *T.mentagrophytes var interdigitale*, WM 07.420, WM07.421, WM 06.838, WM 06.873 were *T.tonsurans*, WM 07.133 T.mentagrophytes var erinacei and WM 06.839 was *T.rubrum*.

This result conforms to that of Jackson *et al.* (1999) although they have reported four patterns in each of two dermatophytes species, ie *T. quinkeanum* and *T. schoenlenii*, *T. soudanense* and *T. rubrum*, *T. equinum* and *T. tonsurans*, and *T. concentricum* and *T. erinacei*. Graser *et al.* (1999) and Kac (2000) have supported the finding of Jackson *et al.* (1999) reporting sequence analysis of the ITS region of *T. equinum* which is surprisingly closely related to the most widespread anthropophilic agent of endothrix *Tinea capitis*, *T. tonsurans*. However, they are easily discriminated by their ecological status as *T. equinum* is usually isolated from horses, while *T. tonsurans* is anthropophilic. Moreover, *T. tonsurans* has abundant microconidia which stain deeply with lactophenol cotton blue and balloon forms of chlamydospores-like cells.

In the laboratory, *T. tonsurans* uniformly shows a distinctive stimulation response to thiamine, and is readily distinguishable from both *T.equinum* varieties in vitamin tests as reported by Kane *et al.* (1997).

In this study, all test isolates of each *Trichophyton* species (*T.mentagrophytes* var *mentagrophyte*, 17 *T. mentagrophytes* var *interdigtale*, seven *T.tonsurans* and two *T.rubrum*) showed an identical profile so an intra species polymorphism was not detected among these isolates using RFLP with *Mva*1 restriction enzyme. This result is in agreement with Mochizuki *et al* (2003) who reported that intra species polymorphism was not detected among twenty six *T. tonsurans* isolates using *Mva*1 and *Hinf*1 restriction enzymes.

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