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Original Article

Application of PCR-RFLP to Rapid Identification of the Main Pathogenic Dermatophytes from Clinical Specimens

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Abstract:

Background: In the present study, a PCR-RFLP based molecular technique was designed to rapid identification of dermatophytes in clinical specimens. Skin scrapings obtained from human cases suspected to dermatophytosis were studied in order to identify involved etiological fungi.

Methods: In this experimental study, the specimens (skin scrapings) of patients referred to Mycology Department of Pasteur Institute of Iran were inoculated on Petri dishes contained selective agar for pathogenic fungi (SAPF) and incubated at 25° C until visible growth of fungal colonies. The colonies were examined for standard morphological characteristics after visible growth on the agar medium. A small portion of each fungal colony was further studied by restriction fragment length polymorphism (RFLP) analysis of the PCR-amplified internal transcribed spacer (ITS) region of ribosomal DNA (rDNA). PCR amplicons were electrophoresed on 2% agarose gel after digesting by different restriction enzymes including *Mval*, *Hinfl* and *HaelII*.

Results: Among 160 clinical samples examined, 6 dermatophyte species including *Trichophyton mentagrophytes, T. ru-brum, T. verrucosum, T. tonsurans, Microsporum canis* and *Epidermophyton floccosum* were finally identified based on the colony morphology and microscopic criteria. Specific PCR products and RFLP patterns for *Mval, Hinfl* and *Hae*III enzymes allowed the rapid identification and reliable differentiation of isolated dermatophytes at the genus or species level for 5-10 day-old colonies.

Conclusions: The results showed that PCR-RFLP analysis of the ITS region of rDNA is a rapid and reliable tool which allows identification of major pathogenic dermatophytes isolated in this study at species level in young 5-10 day-old colonies.

Keywords:

Dermatophytosis , PCR-RFLP , ITS region , Identification , Dermatophyte species

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