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ORIGINAL ARTICLE/ARTICLE ORIGINAL

A comparative study on morphological versus molecular identification of dermatophyte isolates

Étude comparative pour l'identification des dermatophytes : morphologie versus identification moléculaire

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T. rubrum;
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Summary
Objective. — Dermatophytes are taxonomically classified in the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. Pleomorphism, cultural variability, slow growth and sporulation, and the need for additional physiological tests make dermatophytes notoriously difficult to identify. The present study aimed to compare the results of morphological and molecular identification of certain groups of clinical isolates of dermatophytes with a view to evaluating the accuracy of molecular methods.
Patients and methods. — For each sample, the ITS1-5.8S-ITS2 rDNA region was amplified using the primers ITS1 and ITS4. PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis using the enzyme MvaI and isolate identification was performed by comparing the electrophoretic RFLP patterns with reference profiles obtained previously. Finally, paired comparative analyses of molecular and conventional methods were performed.
Results. — While morphology results from routine daily reports of the laboratories indicated that 18 (6.8%) and 136 (52.10%) of the isolates were *T. rubrum* and *T. interdigitale*, respectively, PCR-RFLP results suggested that *T. rubrum* was the most common etiological agent of ringworm accounting for 94 (36.01%), followed by *T. interdigitale* accounting for 71 (27.20%). Interestingly, 80.8% out of the 94 isolates identified as *T. rubrum* by molecular testing had been identified by

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morphological examination as belonging to different species, such as *T. interdigitale* (75.5%), *E. floccosum* (2.1%) and *M. canis*, *T. verrucosum*, and *T. tonsurans* (each 1.06%). Ten strains out of 261 (*T. interdigitale*, *n* = 8; *E. floccosum*, *n* = 2) had been defined as unknown species by morphological tests.
Conclusion. — An unexpected high percent of isolates identified as *T. interdigitale* by conventional methods were in effect *T. rubrum* shown by PCR-RFLP, and regarding the necessity of correct identification of dermatophytes recovered from different clinical forms of the infection, we highly recommend ITS-sequencing or ITS-RFLP of the isolates, particularly for epidemiological research studies.
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MOTS CLÉS
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Résumé Les dermatophytes sont classés en 3 genres toxonomiques : *Trichophyton*, *Microsporum* et *Epidermophyton*. Le pléiomorphisme, les variations en culture, la lenteur de croissance et de sporulation et la nécessité de tests physiologiques complémentaires rendent les dermatophytes notoirement difficiles à identifier.
Objectifs. — Le but de cette étude est de comparer les résultats de l'identification morphologique et moléculaire de certains groupes d'isolats cliniques pour évaluer la justesse des méthodes moléculaires.
Patients et méthodes. — Pour chaque échantillon, la région ITS1-5.8S-ITS2 de l'ADNr a été amplifiée en utilisant les primers ITS1 et ITS4. Les produits de PCR ont été soumis à l'analyse RFLP en utilisant l'enzyme MvaI et l'identification de la souche a été faite en comparant l'échantillon électrophorétique de RFLP aux profils de référence antérieurement obtenus. Finalement, les analyses comparatives moléculaires versus les méthodes conventionnelles ont été réalisées.
Résultats. — Alors que les résultats avec la morphologie indiquaient que 18 (6,8 %) et 136 (52,1 %) des isolats étaient *T. rubrum* et *T. interdigitale* respectivement, les résultats PCR-RFLP suggéraient que *T. rubrum* était l'agent étiologique le plus fréquent pour les lésions cutanées, représentant 94 cas (36,01 %), suivi de *T. interdigitale* 71 cas (27,2 %). Il est à noter que 80,8 % des 94 isolats identifiés comme *T. rubrum* par les tests moléculaires avaient été identifiés par la morphologie comme une autre espèce : *T. interdigitale* 75,5 %, *E. floccosum* : 2,1 % et *M. canis*, *T. verrucosum* et *T. tonsurans* (1,06 % chacun). Dix souches sur 261 (*T. interdigitale n* = 8, *E. floccosum n* = 2) avaient été identifiées comme espèce inconnue par la morphologie.
Conclusions. — Un fort pourcentage inattendu d'isolats identifiés comme *T. interdigitale* par les méthodes conventionnelles étaient en fait *T. rubrum* comme la PCR-RFLP l'a montré. La nécessité d'une identification correcte des dermatophytes isolés des différentes formes cliniques de l'infection nous fait vivement recommander le séquençage ITS ou la PCR-RFLP des isolats en particulier pour les études épidémiologiques.
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Introduction

Dermatophytosis is a fungal infection caused by dermatophytes which are comprised of three genera, *Trichophyton*, *Microsporum*, and *Epidermophyton*, capable of invading keratinized tissues of humans and animals such as skin, hairs and nails [17]. The etiologic agents of infection are distributed worldwide and it is estimated that 20%—25% of the world population have had at least one type of dermatophytosis [7]. The distribution of dermatophytes and dermatophytosis in a given geographical area depends on climate, environmental or socio-economic factors, immigration and, tourism, tends to change over time. The most common dermatophytes in Europe include *Trichophyton rubrum*, *Microsporum canis* and, *Trichophyton interdigitale*; in Asia and Australia, *T. rubrum* and *T. interdigitale*; in Africa, *Microsporum audouinii* and *Trichophyton violaceum* and in the Americas are *T. rubrum*, *Trichophyton tonsurans*, and *T. interdigitale* [13,3].

Correct identification of causative agents of disease is important for epidemiological purposes, control of potential sources of infection, accurate antifungal therapy, prevention of transmission to others and, include exact differentiation between dermatophytosis and non-dermatophyte superficial infections [29,2]. Globally, current baseline dermatophyte species delineation in most laboratories rely on culture-based criteria including micro- and macromorphology of the colony, mating ability, and, biochemical/physiological characteristics [30]. Over the past two decades, the phylogenetic concept of species strikingly revolutionized the taxonomy of dermatophytes, and to ease accurate identification of these fungi, focus has shifted towards culture-independent strategies using molecular methods. These procedures have the advantages of speed, low degree of handling and skill required, and increased sensitivity and reproducibility compared with conventional diagnosis [16]. Apart from the location of study, different frequencies were reported for species in conventional and molecular-based surveys of dermatophytosis [24,31,6].

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