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Review

Molecular epidemiology, phylogeny and evolution of dermatophytes

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ABSTRACT

Dermatophytes are fungi that invade and propagate in the keratinized skin of mammals, including humans, often causing contagious infections. The species of medical concern belong to the genera *Microsporum*, *Trichophyton*, *Epidermophyton* (in their anamorphic state) and *Arthroderma* (in their telomorphic state), which were traditionally identified based on their morphology and biochemical characters. Nonetheless, limitations linked to the differentiation of closely related agents at species and strains level have been recently overcome by molecular studies. Indeed, an accurate identification of dermatophytes is pivotal for the establishment of effective control and prevention programs as well as for determining the most appropriate and effective antifungal therapies to be applied. This article reviews the DNA techniques and the molecular markers used to identify and to characterize dermatophyte species, as well as aspects of their phylogeny and evolution. The applications of typing molecular strain to both basic and applied research (e.g., taxonomy, ecology, typing of infection, antifungal susceptibility) have also been discussed.

Contents

1. Introduction

Dermatophytes are fungi that invade and propagate in the keratinized skin of mammals, including humans, often causing contagious infections (Weitzman and Summerbell, 1995). The disease caused by these fungi (i.e., dermatophytosis) is common worldwide (Outerbridge, 2006) and has veterinary and public health relevance (Cafarchia et al., 2009, 2012; Weitzman and Summerbell, 1995). The distribution of these fungi varies considerably, depending on geographical area of provenience and other epidemiological factors (i.e., age, sex, seasons) (Cafarchia et al., 2004, 2006;

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Iorio et al., 2007; Weitzman and Summerbell, 1995). Dermatophytes belong mainly to the genera *Microsporum*, *Trichophyton*, *Epidermophyton* (in their anamorphic state) and *Arthroderma* (in their telomorphic state) and they include ~30 species (e.g., among the most important, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton mentagrophytes* "complex" as well as *Microsporum canis*, *Microsporum gypseum*, and *Epidermophyton floccosum*) that act as etiologic agents of dermatophytosis in humans (Das et al., 2007; Weitzman and Summerbell, 1995). Based on their ecology, dermatophytes have been divided into three groups, as anthropophile, zoophile, and geophile. Anthropophilic dermatophytes are primarily associated with humans, causing mycoses including *Tinea capitis* and *Tinea corporis* and *Tinea pedis* or onychomycosis, and rarely infect animals (Cafarchia et al., 2006; Gräser et al., 2008; Weitzman and Summerbell, 1995). Zoophilic species are common pathogens of animals, and occasionally infect humans, whereas geophilic dermatophytes are primarily associated with keratinous materials (i.e., hair, feathers and horns) present in the environment, and they might be transmitted to humans and animals through contact with soil (Weitzman and Summerbell, 1995). A precise identification and delineation of isolates at species and strain level is crucial to settle effective programs for controlling and preventing infection and to establish accurate antifungal therapies. For example, in *tinea capitis*, *T. tonsurans* forms arthroconidia inside the hair shaft, where contact with conventional antifungal drugs is relatively high, thus requiring shorter treatment times than *M. canis*, which evades drug exposure by forming arthroconidia outside the hair shaft (Gupta et al., 1999).

Until recently, identification of dermatophyte species has been essentially performed by a combination of microscopic approaches and in vitro-culture by colony morphology and other features of conidia examination (de Hoog et al., 2000; Rebell and Taplin, 1979). Identifications are often complicated and laborious due to the morphological similarities shared between species, it is time consuming and requires a high level of scientific knowledge and training (Gräser et al., 2008; Kanbe, 2008; Nenoff et al., 2013). The chemotaxonomic methods (i.e., disc electrophoresis of culture filtrate proteins, pyrolysis–gas–liquid chromatography to study fatty acids, polyacrylamide gradient gel electrophoresis of total cell protein extracts for zymogram patterns, isoelectric focusing of somatic extracts in thin-layer polyacrylamide gels and Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) have been developed to bypass the traditional microscopic identification of cultivated dermatophyte strains but are often inaccessible to conventional laboratories (Nenoff et al., 2013).

Since the early 80s (Davidson et al., 1980) many molecular techniques have been developed for identification of dermatophytes at species or strain level (Gräser et al., 2008; Kanbe, 2008; Nenoff et al., 2013). As a consequence, some biotypes, unambiguously considered as species in the past, were not distinguishable molecularly (Gräser et al., 2008) and *vice versa*, leading to confusion in the traditional taxonomical classification for this group of fungi and to the proposal of a novel classification (Gräser et al., 2008). Here, we review the DNA techniques and the molecular markers used to identify and characterize dermatophytes, and discuss aspects of their phylogeny and evolution. The advances in molecular typing have also been instrumental to a better understanding of their taxonomy, ecology and epidemiology.

2. History of taxonomy of dermatophyte

In the mid-19th century, Robert Remak observed peculiar microscopic structures (Remak, 1842), which were previously associated to fungal species (Schoenlein, 1839), and named them *Achorion schoenleinii* (Remak, 1845). Following the first description of *Microsporum audouinii* and *Herpes* (*Trichophyton*) *tonsurans* from cases of human *tinea capitis* (Gruby, 1843, 1844; Malmsten, 1845; Robin et al., 1853), the dermatophytes were classified into four genera, *Achorion*, *Epidermophyton*, *Microsporum*, and *Trichophyton* based on a combination of clinical presentation, cultural and microscopic observations (Sabouraud, 1910), and the agents of favus-like diseases were included within the genus *Achorion* (i.e., *Trichophyton schoenleinii*, *T. mentagrophytes sensu stricto*, *Microsporum gallinae* and *M. gypseum*).

Based on the vegetative structures and conidia (i.e., *fungi imperfecti*), the original taxonomic scheme was revised, and the genus *Achorion* was eliminated, while the taxonomical status of only three genera (*i.e.*, *Microsporum*, *Trichophyton*, and *Epidermophyton*) was confirmed and the number of valid species was reduced to 19 (Emmons, 1934).

In addition, the identification of this group of pathogens was refined based on nutritional and physiological features, which led to the unification of *T. tonsurans* varieties and the recognition of *Trichophyton equinum* as a proper agent (Georg and Camp, 1957; Swartz and Georg, 1955). The discovery of the teleomorphic state of dermatophytes (perfect or sexual state), introduced the concept of biological species, further complicating the taxonomy of dermatophytes (Dawson and Gentles, 1961; Griffin, 1960; Kane et al., 1997; Rebell and Taplin, 1970). Indeed, according to their teleomorphic state, the genus *Microsporum* was initially classified into *Nannizzia* and later unified into *Arthroderma* (Weitzman et al., 1986) in which also *Chrysosporium*, *Keratinomyces* and *Trichophyton* were included. The genera *Epidermophyton*, *Microsporum, Trichophyton, Keratinomyces* and *Chrysosporium* (the latter two comprising manly non-pathogenic fungi) were recognized based on their anamorphic characters. The *Keratinomyces* genus was synonymised with *Trichophyton* by Ajello (1968). Additionally the anamorphic characters of *Keratinomyces ajelloi* (i.e., the presence of micro and macroconidia) suggested its close association with *Trychophyton* genus (de Hoog et al., 2000). On the basis of mtDNA restriction analysis *K. ajelloi*, *Keratinomyces ceretanicus* and *Keratinomyces longifusus* were recognized as different species but only a limited set of reference species of the genus *Trichophyton* was analysed to confirm the close association between the genera (i.e., *Keratinomyces* and *Trichophyton* – Guillamón et al., 1996). A phylogenetic analysis, showing the relationship between higher-level taxonomy (e.g., genera) was not done either.

Today, dermatophytes are also classified into geophilic, zoophilic and anthropophilic species according to their development in soil, on animals or humans, respectively (Ajello, 1962; Georg, 1960).

Since 1980, improved knowledge of the genetic make-up of nuclear and mitochondrial genes of dermatophytes and the advent of molecular methods (e.g., RFLP, fingerprinting techniques and sequencing technologies), have contributed greatly towards the understanding of the biodiversity of dermatophytes, thus challenging the previous classification (Davidson et al., 1980).

For example, sequencing of the internal transcribed spacer of ribosomal DNA (ITS rDNA) allowed to group dermatophytes based on their clinical and ecological traits, rather than their morphological features (Gräser et al., 1999a). Indeed, phylogenetic reconstructions of ITS sequences support the separation of geophilic species from the remaining members of the Arthrodermataceae, which were referred as the 'true dermatophytes'. The little information available on mating features for some species (i.e., mammal-associated dermatophytes), highlighted the importance of phylogenetic studies in the definition of species which led to a reduction of dermatophyte species or varieties (see Tables 1 and 2). Accordingly, a new classification of anthropophilic and zoophilic dermatophytes was proposed (Table 3, Gräser et al., 2008).

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