The application of MALDI-TOF MS for dermatophyte identification

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ABSTRACT. Dermatophytes are keratinolytic fungi responsible for a wide variety of diseases of the skin, nails and hair of mammals. Their identification is often complicated, labor-intensive and time-consuming due to the high degree of intra-species morphological similarity, and also requires scientific knowledge and practice. The aim of this study was to demonstrate that MALDI-TOF MS technique may be a faster and more sophisticated method useful for the identification of dermatophytes and mycoses in general.

Key words: dermatophyte detection, Matrix-assisted laser desorption ionization-time of flight mass spectrometry, MALDI-TOF MS

Dermatophytes and yeasts are the major etiological factors of superficial fungal infection in human and animals. Dermatophytes cause a number of infections, such as dermatophytosis or ringworm, in both healthy and immunocompromised patients [1]. These superficial mycoses affect 20% to 25% of the global population [2]. An accurate etiologic diagnosis of dermatophytes is important, not only to guide clinical management, but also for epidemiological purposes.

The dermatophytes are a closely-related and highly-specialized group of unique pathogenic fungi. They have the ability to invade the stratum corneum of the epidermis and keratinized tissues derived from it, such as hair, nail and skin in both humans and animals [3]. Taxonomically, these filamentous fungal pathogens are classified into three genera, Microsporum, Trichophyton and Epidermophyton, which cause infections only in humans.

The routine, conventional laboratory diagnosis of dermatomycoses consists of the direct microscopic examination of clinical specimens followed by in vitro culture techniques. However, direct microscopic identification of fungal elements in clinical samples, using potassium hydroxide (KOH), may be a quick method, but its specificity and sensitivity are low and are dependent on the experience of the researcher. Moreover, false negative results are possible in 5% to 15% of cases [4,5]. In contrast, identification based on in vitro culture is a more specific but time-consuming diagnostic test [1], taking up to 4 weeks or longer to give results, and intra-species morphological polymorphism and phenotypic pleomorphism may sometimes be confusing [3,6].

In the past few decades, as microorganisms have become more resistant, difficult to culture and identify, researchers have looked to molecular diagnostics and proteomic technologies for solutions, resulting in the use of technologies new to Microbiology [7]. Such technologies include polymerase chain reaction (PCR) technology, DNA microchips, sequencing technology, mass spectrometry, immunoassay testing, and molecular hybridization probes. These molecular technologies hold the key to keeping pace with the increasing number of agents that threaten public health. They facilitate the fast and reliable identification of both dermatophytes and microorganisms in general. In
recent years, many techniques based on PCR, such as PCR fingerprinting, Random Amplification of Polymorphic DNA (RAPD) [8], Restriction Fragment Length Polymorphism (RFLP) [9] and real-time PCR [10], have been used to identify the genera or species of dermatophytes. The fragments of DNA required for identification usually contained the genes encoding DNA topoisomerases II [11] or chitin synthase I [1], although other DNA fragments employed in these studies are 28S ribosomal DNA (rDNA) [12] or internal transcribed spacer region 1 (ITS1) [8,10].

Another new sophisticated method used in mycological diagnostics is matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS is now used routinely in clinical diagnostic laboratories, as it is faster than PCR and requires little sample handling. This is a new approach to microbial identification and is based on characteristic fingerprints of intact cells. The basis of this technique is the generation of a spectral profile which is specific for the isolate and largely determined by ribosomal protein content. The acquired protein spectra are subsequently compared to an extensive reference library of spectra using various types of analytical software to give a list of top-matching identifications. The method is regarded as a cost-effective and reliable technique for the identification and typing of many microbial pathogens [13,18], and has already been validated for rapid and accurate identification of bacterial, yeast, and mould species [14–16]. However, only a few studies have evaluated the use of MALDI-TOF MS for the identification of dermatophyte fungi [13,17–22].

Theel et al. [18] compared MALDI-TOF with 28S rRNA gene sequencing as methods for the identification of dermatophytes from clinical cultures. The method was found to represent a fast, cost-effective and highly-specific technique for the differentiation of dermatophyte species grown in culture. Misidentification at the genus level occurred only once with a single isolate of *Epidermophyton floccosum*. However, the disadvantage of this technique is that it depends on the choice of database. The results differ significantly based on whether they were obtained from the standard Bruker library (MBL) alone, or from the S-MBL: an MBL library supplemented with 20 additional dermatophyte spectra from clinical isolates (identified by D2 28S rRNA sequencing) and 4 American Type Cell Culture Collection (ATCC) strains. Although MALDI-TOF correctly identified 22.2% of isolates to the genus and 4.7% of isolates to the species level when the MBL was used, the use of the S-MBL improved isolate recognition to 86.0% at the genus level and 36.8% at the species level. Presumably, these results differed because the S-MBL library included an additional nine dermatophyte species, four of which (*Microsporum audouinii, M. persicolor, Trichophyton soudanense, T. verrucosum*) were not represented in the MBL. These results demonstrate that the libraries used in this technique should be regularly expanded and updated. Nevertheless, this method is an alternative to traditional or molecular methods for dermatophyte identification [18,19].

De Respinis et al. [13] tested MALDI-TOF MS as a technique for the identification of the most important dermatophyte species. The analysis was made after 3 days of incubation of clinical isolates. All strains were characterized by morphological criteria and ITS sequencing (gold standard). The dendrogram resulting from MALDI-TOF mass spectra was almost identical with the phylogenetic tree based on ITS sequencing. Overall, 95.8% of the clinical isolates were correctly identified by MALDI-TOF MS. According to the authors, this method allowed closely related and morphologically indistinguishable species to be discriminated.

Alshawa et al. [20] used MALDI-TOF MS with the Andromas system to identify 12 dermatophyte species, however, the sample did not include some important ones, like *Microsporum audouinii, M. gypseum* or *Trichophyton verrucosum*. This method obtained comparable results for correct species level identification (91.9%) to those of a previous work by De Respinis et al. [13]. However, their method required far more time, 3-week-old cultures were used for investigation, which makes this method unsuitable for diagnostic use.

Another application of MALDI-TOF MS for dermatophyte identification is presented by Hollemeyer et al. [21]. This method is rapid and does not require any cultivation. The mass spectra of tryptic digests of samples infected by *Trichophyton rubrum* were found to be appreciably different from those of healthy persons and of patients suffering from psoriasis. The results of the study also showed that the homogeneity of the spectra within the group of samples infected with *T. rubrum* was not as high as within either of the other
two groups: the healthy and psoriasis-affected patients. According to Hollemeyer et al. [21], this indicates a progressive degradation of structural proteins during the progression of the fungal infection.

The currently-used MALDI-TOF MS techniques are nearly independent from culture conditions. Since small amounts of material are enough for proper analysis, sample preparation is an important factor contributing to the quality of analysis. In some instances, such as in fungi, a strong cell wall may be present in the sample and extraction may be required to render ribosomal proteins available for analysis.

MALDI-TOF MS is increasingly used for microbiological diagnostics and has already replaced conventional biochemical identification, but minor discrepancies are observed between these methods. MALDI-TOF MS pathogen identification is based on the analysis of ribosomal protein spectra, and such results are closely related to the results of 16s rDNA sequence database comparisons. Species which do not differ sufficiently in their ribosomal protein sequences cannot be distinguished by this method [22].

In conclusion, MALDI-TOF MS seems to be an excellent alternative to microscopy, PCR and sequencing for dermatophyte identification. However, it must be noted that it is still necessary to expand the databases of reference spectra for each species, to sufficiently encompass intra-species strain diversity. One way for users to compare spectra for isolates and enlarge their own reference database would be to establish an open source platform.

References


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