Microbial Diagnostic Applications of Mass Spectrometry

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ABSTRACT BOOK

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NEW INSIGHTS FOR IDENTIFICATION OF CLINICAL ISOLATES OF *Trichophyton rubrum* USING MALDI-TOF MS

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Dermatophytoses are the most common fungal infection worldwide with a non-despicable impact in health-care costs. *Trichophyton rubrum* is an antropophilic dermatophyte species very well adapted to human host causing chronic and slowly progressing disease on keratinised tissues. It is the causative agents of about 70% of all human dermatophytoses. Besides their distribution all over the world this species is by far, the most frequently isolated species on onychomycosis and tinea pedis. Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS) analysis has already been used as a rapid technique in the identification and classification of microorganisms. It has progressively been incorporated as a technique in the polyphasic approach to improve the accuracy of the microbial identification issue. This technique has also been used as a tool for the fast identification of filamentous fungi with clinical relevance including dermatophytes. In this study twenty clinical dermatophyte isolates were analysed using a polyphasic approach that was based on macro- and micro-morphologies, biochemistry, molecular biology using ITS 1-4 sequencing data and primers M13, $(GACA)_4$ and $(AC)_{10}$ for typing and, MALDI-TOF MS analyses. Eighteen of these clinical dermatophyte isolates were collected from human nails. The remaining 2 isolates were the reference strain *T. rubrum* ATCC MYA-4438 that was used as positive control and the strain *T. mentagrophytes* ATCC MYA-4439 that was used as out-group. Preliminary results based on macro- and micro-morphologies indicated that all isolates were *T. rubrum*. These results were confirmed by molecular biology and MALDI-TOF MS techniques. For molecular approach 16 *T. rubrum* isolates were clustered in a single group with 100% of genotypic homology of the ITS1-4 region. Moreover, 2 remaining *T. rubrum* isolates were found having 98% of homology in this region. Similar clusters were found for M13 and $(GACA)_4$ primers. In contrast, $(AC)_{10}$ was not discriminative. MALDI-TOF MS analysis corroborates with morphological and molecular identifications. Nine *T. rubrum* isolates were clustered on a single group evidencing 100% similarity and the remaining *T. rubrum* isolates were distributed over the MALDI-TOF MS dendrogram showing phenotypic variability. MALDI-TOF MS shown to be as good as molecular biology and, moreover, rapid, low-cost and accurate alternative tool for identification and strain typing of *T. rubrum* clinical isolates. Analysis of mass spectra profiles provided new insights in the proteomic approach for strain typing of clinical isolates of *T. rubrum*. As a matter of consequence, MALDI-TOF MS can be suitable as point-of-care diagnostic for dermatophytoses.

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