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## MALDI-TOF ICMS vs molecular typing in the identification of dermatophyte strains

Leonel Pereira<sup>1</sup>, Nicolina Dias<sup>1,2</sup>, Cledir Santos<sup>1</sup>, Nelson Lima<sup>1</sup>

<sup>1</sup>IBB-Biological Engineering Center, Micoteca da Universidade do Minho, Braga, Portugal

<sup>2</sup>CITS - Centro de Investigação em Tecnologias da Saúde, Paredes, Portugal

nelson@ie.uminho.pt

Dermatophyte infections are the most common fungal infection worldwide with a non-despicable impact in health-care costs. Dermatophytes belong to the group of parasitic fungi with affinity for keratinised tissues, able to infect skin, nails and hair of humans and animals. However the infection remains in the *stratum corneum* of the epidermis and reports of systemic disease caused by those agents are very rare and restricted to immunocompromised patients. Antropophilic species are very well adapted to human host causing chronic and slowly progressing disease although zoophilic species produce acute inflammation upon infection. Therefore antropophilic species are the causative agents of about 70% of all human dermatophytoses. Among those, *Trichophyton rubrum* is, by far, the most frequently isolated species on onychomycosis and tinea pedis all over the world (White *et al.* 2008).

Identification of individual species remains important from an epidemiological point of view and also for therapy administration. Phenotypic characters and ecological features have been for long time the basis for phylogenetic tree establishment and taxonomic classification. However pleomorphism and cultural variability make identification a very hard task. Physiological and biochemical tests have been developed to complement identification process. Molecular phylogenetic analysis provided microbiologists with powerful tools for accurate organism identification and lead to the reorganization of taxa (Gräser *et al.* 2000; Gräser *et al.* 2008), solving problems concerning morphology-based identification of dermatophytes and improving knowledge on the epidemiology of dermatophytes (Kanbe 2008). Recently a spectral technique analysis by Matrix Assisted Laser Desorption Ionization Time of Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) has been increasingly used as a rapid technique in the identification and classification of microorganisms. It has proved also to be a valuable tool being incorporated in the polyphasic approach to improve the accuracy of the microbial identification issue.

In this study, nineteen clinical isolates of *T. rubrum* from human nails were analysed using a polyphasic approach that was based on macro- and micro-morphologies, molecular biology using Trubrum-for (5'-TCTTTGAACGCACATTGCGCC-3') and Trubrum-rev (5'-CGGTCCTGAGGGC GCTGAA-3') primers and MALDI-TOF ICMS analyses. All *T. rubrum* identifications were confirmed by morphological, molecular and spectral techniques. Additionally, eight isolates (ND0004, ND0005, ND0007, ND0022, ND0032, ND0033, ND0035 and ND0044) were grouped together as “clones” by MALDI-TOF ICMS evidencing 100% identity in the MALDI dendrogram (Fig. 1).

In order to clarify if spectral results have any correspondence to the molecular data, primer M13 (5'-GAGGGTGGCGGTCT-3'), (GACA)<sub>4</sub> (5'-GACAGACAGACAGACA-3') and

(AC)<sub>10</sub> (5'-ACACACACACACACACACAC-3') were used as fingerprinting tool for strain typing of these isolates. Fig. 2 shows the results of PCR fingerprinting using (GACA)<sub>4</sub> primer for 8 *T. rubrum* isolates.

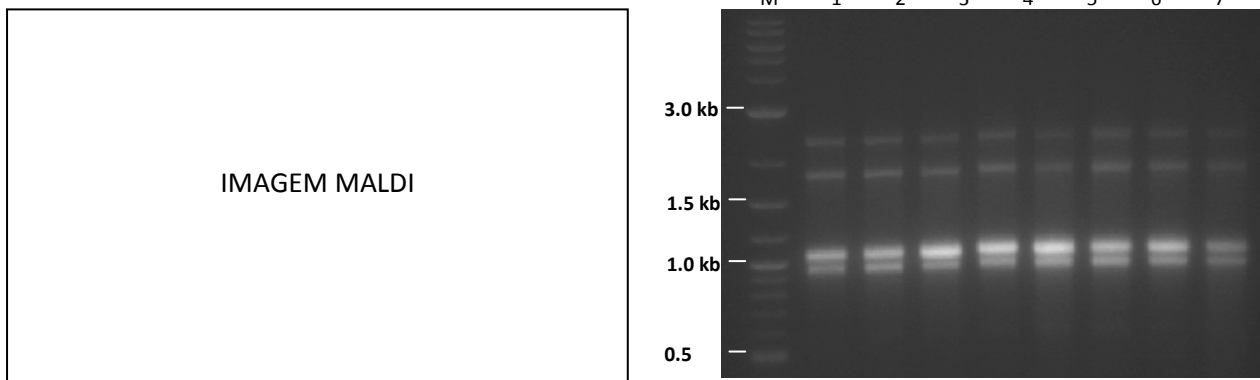


Figure 2 - *T. rubrum* PCR fingerprinting patterns obtained with primer (GACA)<sub>4</sub>. Lane M, 0.1-10 kb ladder; lane 1, ND0004; lane 2, ND0005; lane 3, ND0007; lane 4, ND0022; lane 5, ND0032; lane 6, ND0033; lane 7, ND0035; lane 8, ND0044.

Surprisingly, the molecular biology data obtained from 3 different primers corroborate those found by MALDI-TOF ICMS concerning the occurrence of 8 “clones”. Both approaches have shown the same accuracy in typing dermatophytes.

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