

Classification of dermatophytes by a multilocus phylogenetic approach based on *Tef-*1 α , β -tubulin and *ITS* genes

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Objectives

Identification of dermatophytes to the species level is epidemiologically, ecologically and therapeutically significant. The use of phylogenetic species concepts based on rDNA internal transcribed spacer (*ITS*) regions have improved the taxonomy of dermatophytes. However, it has been shown that for some species, confirmation and refinement using other genes are needed. Indeed, there are problems for relevant definition and characterization of dermatophytes especially among the *Trichophyton mentagrophytes* series. Intra- and interspecies variations of the translation elongation factor 1- α (*Tef-1a*) gene were evaluated as a new identification marker in a wide range of dermatophytes. Therefore, the aim of this study was to evaluate the discriminatory power of a phylogenetic tool based on **concatenated sequences of three genomic regions including** *Tef-1a*, *ITS* (*ITS1* to *ITS2*) and β -tubulin to differentiate: 1/ the anthropophilic *Trichophyton interdigitale* species from the zoophilic *Trichophyton mentagrophytes* 2/ the two closely related African anthropophilic species *Trichophyton violaceum* from *Trichophyton soudanense*

Methods

- ITS1-2/Tef-1 α / β-tubulin
- 26 strains of *T.interdigitale/T.* mentagrophytes
 31 strains of
- T.soudanense/T.violaceum

sequencing + Real time PCR DermaGenius (Pathonostics) for confirmation of the identification

- Alignments with MUSCLE program (Seaview)
- Concatenation of the 3 genes
 - Phylogenies inferred on the concatenated alignment using PhyML.

Results

1) T. interdigitale /T. mentagrophytes

After generation of the phylogenetic tree by concatenation of the three sequence genes, the differentiation between T. interdigitale and T. mentagrophytes was clear. The 15 strains of T. interdigitale were well classified into one distinct group on the dendrogram. The 11 strains of T. mentagrophytes were well distinct of the anthropophilic group and defined by another clade on the dendrogram. Reference strains were correctly classified. Among the 11 T. mentagrophytes strains, only one strain (190225-0016) has been identified by DermaGenius (DG) as T. mentagrophytes, the other strains of this group being identified as T. interdigitale by this method. This shows that DG is not accurate for the differentiation between T. interdigitale and T. mentagrophytes. Using this kind of phylogenic tree, can help for the differentiation of these two closely related species of dermatophytes.

2) T. soudanense/T.violaceum

- The concatenation allowed to define two well distinct clades on the dendrogram. One clade containing 10 *T. violaceum* tested and the other containing the 20 *T. soudanense* included in the study. Reference strains were correctly classified into each corresponding clade.
- However, one strain the "180306-0068", that has been identified as *T. violaceum* by DermaGenius and microscopy, is classified among the *T. soudanense* strains. This traduces a high similarity between the tree genomic regions tested for these two species. Only one mutation can move the position of the strain on the tree what is not optimal. Other candidates genes should be added to better increase the differentiation between these two species such as, based on the literature, *LSU*, *TEF3*, or *RPB2*.



Conclusions

The concatenation of *ITS*, *B*-tubulin and *Tef*-1a genes sequences allows the generation of a discriminant dendrogram between the zoophilic *T*. *mentagrophytes* and the anthropophilic *T*. *interdigitale*. This is less discriminant between the two closely related African anthropophilic species *T*. *violaceum* and *T*. *soudanense*. Other candidates genes should be evaluated to increase the discriminatory power of the philogenetic tree for these two later species. Anyway, this multilocus phylogenetic approach allows to better define the species boundaries between dermatophytes and facilitates the molecular characterization of species in routine diagnostic.