PRP8 intein applied to identification and phylogeny of dermatophytes.

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INTRODUCTION

Inteins are self-splicing protein elements found in some housekeeping genes important for cell survival. They can be found as a full-length intein when they have a splicing and a homing endonuclease (HE) domains or as a mini-intein when only the splicing domain is present¹. The PRP8 intein is inserted into the PRP8 protein, a core protein of the spliceosome complex responsible for removing introns during the splicing process². It was observed that variations in the nucleotide sequences of the PRP8 intein reflects the phylogenetic relationships of fungal species and its longitude polymorphism enables a proper molecular identification by electrophoresis techniques^{3,4}. Since a PRP8 intein was by us detected in dermatophytes **we aimed** to determine the phylogenetic relationship based in PRP8 intein and to identify clinically important *Microsporum* and *Nannizzia* species by amplification and visualization of some polymorphic regions (HE region) with no need of sequencing.

METHODS

- 40 strains, Brazilian culture collections. Eleven dermatophytes species.
- Molecular identification by sequencing the ITS1-5.8S-ITS2 and D1/D2 rDNA region.
- Primer Design for intein sequencing: **EXTDF** 5' ATCAAGYTG CACTG GAA AC 3' / **EXTDR** 5' GACAAGTCARAGACATGA TGG 3' (extein regions) and **INTDF** 5' TCCTKGGRCCTYTGGC TTGG 3' and **INTDR** 5' CGGACRGYRCGCGAATTG 3'.
- PCR/Purification and sequencing of PRP8 intein by Sanger Method.

***Microsporum* and *Nannizzia* identification by electrophoresis assay.** **HE1F** 5'TTCCTKGGRCCTYTGGCTTGG 3' / **HE2R** 5'ADYAAACCDGCRAGGACGG3' (for *Microsporum* identification) and **HE3R** 5'GTCCAG ATCRYCCWCTTTSG 3' (For *Nannizzia* identification). Agarose gel 2%. 2h, 120V.

RESULTS

Figure 2: Phylogenetic construction using DNA sequences of the PRP8 intein in dermatophytes. The evolutionary history was inferred by using the Maximum Likelihood method

Figure 3: Electrophoresis of PCR products from PRP8 intein HE region for identifying *M. audouinii* and *M. canis*. **A)** HE1F/HE2R PCR products of *Microsporum* genus. Lanes 2 to 11 correspond to *M. canis* (strain 747), *M. canis* (strain 841), *M. audouinii* (strain 483), *N. incurvata* (strain 586), *N. incurvata* (strain 589), *N. gypsea* (strain 546), *N. gypsea* (strain 613), *N. persicolor* (strain 1019), *N. fulva* (strain 1025) and negative control, respectively. **B)** Electrophoresis of clinically important species of *Microsporum* and *Trichophyton* genus. Lane 2 to 8: correspond with *M. canis* (strain 841), *M. audouinii* (strain 483), *N. incurvata* (strain 589), *N. gypsea* (strain 546), *T. rubrum* (strain 31), *T. interdigitale* (strain 28) and negative control, respectively. Lanes 1 and 12 of Figure 1 A and lanes 1 and 9 of Figure 1 B correspond with the 100 bp Molecular Marker (Thermo Scientific, Carlsbad, USA).

Figure 2: Electrophoresis of HE1F/HE3R PCR products from PRP8 intein HE for identifying *A. gypseum* complex species. Lanes 2 to 5 correspond to *N. incurvata* (strains 589, 586, 541, B15); lanes 6 to 8 correspond to *N. gypsea* (strains 613, 435, 546); and lanes 9 and 10 correspond to *N. persicolor* (strain 1019) and *N. fulva* (strain 1025), respectively. Lanes 1 and 11 contain the 100 bp Molecular Marker (Thermo Scientific, Carlsbad, USA) and lane 12 the negative control.

Conclusion

- All dermatophyte studied species have a full-length intein with a HE of the LAGLIDAD family.
- The PRP8 intein is a excellent molecular marker for identification and phylogenetic studies in dermatophytes.
- Some medical important *Microsporum* and *Nannizzia* species might be identified by PCR/Electrophoresis assay.

References

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