Molecular tools for gene deletion in *Fonsecaea pedrosoi*

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1. Background

Fonsecaea pedrosoi is the main etiological agent of chromoblastomycosis (CBM), a disease with a worldwide distribution, but prevalent in tropical and subtropical countries. It is a filamentous fungus with hyphae and conidia pigmented due to the production of melanin. The biology, virulence factors and mechanisms of pathogenesis of *F. pedrosoi* are not explained and therefore studies involving molecular tools for genetic deletion are essential to improve knowledge about the disease and potentially develop new antifungal therapies. One of the most widely used techniques for genetic transformation in other fungi is biolistics, a technique widely used to deliver genetic material directly to cells and intact tissues of non-lethal form through the bombardment of particles. In this study, the main objective was to use strains of *F. pedrosoi* (CBS 271.37) to generate auxotrophic mutants for tryptophan (TRPS), constructing a double-joint PCR deletion cassette and bombarding particles.

2. Material and Methods

1. Cassette construction

Gene deletion cassettes were constructed using the double-joint PCR technique (Kim et al., 2008) (figure B). The gene chosen for deletion was TRPS (Tryptophan synthase – figure

2. Biolistic transformation (figure C)

3. Results

Confirmation of the deletion in the *F. pedrosoi* genome by PCR (E). The gDNA from selected transformants was used to amplified by PCR with primers specific for HPH (internal) and TRPS (external) with expected fragment of 2.5 kb and 2.4 kb. CBS 271.37 gDNA was used as control (WT).

Figure D. HybB-resistant mutants transformed by biolistic after growth in selective and non-selective medium. Sabouraud agar was used with or without Hygromycin B / Tryptophan (50 µg / mL- 500 µg / mL), incubated at 25 ° C for 7-10 days. The blue square indicates the mutants selected for PCR confirmation.

4. Conclusions

Deletion of genes using biolistic and PCR double joint is well characterized in other fungi, such as *Aspergillus sp.* and *Cryptococcus sp.* The experiments demonstrated that the use of biolistic and DJ PCR for the genetic transformation of *F. pedrosoi* through conidia is efficient. Mutants are able to grow in media containing antibiotics and maintain mitotic stability. Particle bombardment is a fast and advantageous method and with this study it is possible to study several genes of *Fonsecaea* to better understand the pathobiology, since the adjustment of protocols and use of molecular tools for genetic deletion is being described for the first time.

5. References

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