Functional characterization of Urease (ureA) in *Fonsecaea pedrosoi*, the causative agent of Chromoblastomycosis

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Abstract

Fonsecaea pedrosoi, the main agent that causes chromoblastomycosis (CBM) in humans, is a filamentous fungus with a worldwide distribution. CBM is prevalent in tropical and subtropical regions, with high incidence in Africa and Latin America. The biology, virulence factors and mechanisms of pathogenesis of *F. pedrosoi* are still poor understood and for this reason studies involving the mechanisms this fungal pathogen uses to stablish the infection are essential for the improving the knowledge about the disease and potentially develop new antifungal therapies. Recently our group described two genetic transformation methods to facilitate the gene function studies in this fungus. We use biolistic transformation to knock out *F. pedrosoi* ureA gene (coding for Urease) by homologous recombination and gene replacement. The main objective of this work was to characterize *F. pedrosoi* urease and evaluate its potential role on virulence of this fungal pathogen.

Methodology

Cultivation of *F. pedrosoi* on Potato Dextrose Broth for 7 days/ 28° C

Separation of the conidia

Biolistic transformation of cassette deletion

Selection for HygR transformants

Screening on Christensen's Urea Agar for urease negative transformants

Genomic DNA extraction

PCR to confirm HygR integration on the urease locus

Phenotypic characterization of the knock out mutants

Results

This work describes, for the first time, the literature to date, the construction and obtaining of a *F. pedrosoi* mutant by homologous recombination and gene deletion. The gene encoding the enzyme urease is well characterized in other fungi such as *Cryptococcus sp.* and *Coccidioides posadasii* as a factor of fungal virulence. In *F. pedrosoi* the absence of the ureA gene was confirmed by the inability of the mutant ΔureA to produce urease in Christensen's Urea Agar. The mutant was able to grow in culture medium with different nitrogen sources but showed a growth defect when urea was the only source of nitrogen. No growth defects were observed on the stress conditions evaluated (data not shown). *In vitro* virulence experiments are underway to date (data not shown).

Figure 2- Confirmation of urease deletion in *F. pedrosoi*, the deleted mutants are indicated on the figure as well as the expected size of the fragments. The controls were genomic DNA of CBS271.37 and H₂O.

Conclusion

For the first time in literature to date, a knock out mutant was constructed in *F. pedrosoi*, which is one of the main causative agent of CBM. The ΔureA is under phenotypic characterization and the data generated by this work will potential identify the role of urease on *F. pedrosoi* virulence traits. This work opens new perspectives for fungal pathogenesis studies by gene deletion in this organism.

Financial support

Figure 1- (A) Multiple alignments of the putative urease amino acid sequences from different organisms: *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Cryptococcus gattii*, *Helicobacter pylori*, *Sporothrix schenckii*, *Sporothrix brasiliensis*, *Cyphellophora europaea*, *Exophiala dermatitidis*, *Cladophialophora carionii*, *Fonsecaea multimorphosa*, *Fonsecaea pedrosoi*, *Fonsecaea rubica*, *Fonsecaea erecta*, *Penicillium digitatum*, *Coccidioides posadasii*, *Coccidioides immitis*, *Rhinocladiella mackenziei*, *Paracoccidioides lutzii*, *Paracoccidioides brasiliensis* performed in CLUSTALW server. In yellow are the three histidine required to: (I) binding to the nickel metalocenter, binding to the substrate (II), and catalysis (III). (B) Cladogram highlighting the genetic relatedness among the urease proteins of Fonsecaea species. It was used the CLUSTALW to create an unrooted tree based on the Neighbor-joining Method (Mol. Biol. Evol. 4: 406-25)