Transcriptional regulatory control of *Histoplasma capsulatum* for biofilms formation

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INTRODUCTION

Histoplasma capsulatum variety *capsulatum* is responsible for a human systemic mycosis that constitutes a major health problem in the world, named histoplasmosis (Rodríguez-Cerdeira *et al.*, 2012). Some microorganisms attached to biological and nonbiological surfaces are able to form biofilms, which are dynamic communities enclosed in an exopolymeric matrix (Costerton *et al.*, 1995). Biofilm formation by *H. capsulatum* was described by our group as a new virulence factor (Pitangui *et al.*, 2012; Pitangui *et al.*, 2016).

OBJECTIVE

Based on the importance of biofilms and its persistence on host tissues and cell surfaces, the present study was designed to investigate the transcriptional regulation of *H. capsulatum* biofilms and planktonic cells.

METHODS

H. capsulatum biofilms and planktonic cultures were developed *in vitro* using EH-315 (bat clinical strain) and 60I (human clinical strain) strains. Complete sequencing of the transcribed regions was performed by HiSeq Illumina platform including construction of cDNA libraries, cluster generation and sequencing (Fig. 1). The data obtained were analysed by software Seqclean 1.9.9 – Next Generation Sequencing Cleaning and "de novo" assembly was performed from the sequencing data using CLC Genomics Workbench 6.5.1. software.

Fig. 1. Experimental workflow for RNA-Seq using the Illumina HiSeq 2500. Basic steps of Next Generation Sequencing (NGS) method which comprises sample/library preparation, cluster amplification, DNA sequencing, image analysis/base calling, read alignment, and variant discovery.

RESULTS

The results obtained show that the conformation of yeasts in biofilms induces a negative regulation of the transcriptional processes resulting from repressed expression of most genes ($\approx 80\%$ down-regulated) (Fig. 2). In addition, the identification of the transcripts products with significantly altered expression in biofilms reveals that proteins with hydrolase activity (cell wall-associated hydrolase) and an elongation factor (EF 1-alpha) appear to play fundamental roles in structuring this pathogen in complex three-dimensional networks which characterize biofilms (Fig. 3).

Fig. 2. Transcriptional regulatory control of *H. capsulatum*. Number of up-regulated and repressed (down-regulated) transcripts, obtained by a transcriptomic approach. Differential transcriptomic profile of the strain EH-315 in biofilms and planktonic growth (A), and 60I strain in biofilms and planktonic growth (B). DET - Differentially expressed transcripts.

Fig. 3. Fold Change Plot of differentially expressed transcripts in *H. capsulatum* biofilms vs. planktonic cultures, EH-315 (A) and 60I (B) strain. Representation of 20 transcripts with significant differential expression and functional annotation (Gene Ontology - GO).

CONCLUSION

In the present study are indicated the molecules (transcripts) that constitute a potential source to delineate: a) a marker of biofilms; b) new prototypes for the therapy of histoplasmosis and; c) new biomarkers as diagnostic tools for histoplasmosis.

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