TITLE: CRISPR/CAS9-MEDIATED GENE DISRUPTION SYSTEM DEVELOPMENT: VECTOR TARGETING *Histoplasma capsulatum*

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ABSTRACT:

Histoplasma capsulatum is the causative agent of the systemic mycosis histoplasmosis, being quite relevant for worldwide mortality and morbidity. Dimorphic fungal pathogens have as prevalent non-homologous or illegitimate recombination, what frustrate gene deletion attempts. The CRISPR/Cas9 system of bacteria has been developed for targeted mutagenesis of eukaryotic genomes with high efficiency and through a mechanism independent of homologous repair machinery. In this work a new plasmid was designed based on the approachs of Nodvig *et al* and Klein *et al* witch have been successful targeting fungi such as *Aspergillus* and *Blastomyces* species. A T-DNA Binary vector, with kanamycin resistance gene and border repeat sequences, necessary for Agrobacterium-mediated gene transfer, was used as backbone. The construction was worked to disrupt the hsp60 gene, which translate to the heat shock protein 60. This chaperone has been described as an essential surface molecule, mediating the recognition and phagocytosis of the yeast by macrophages. The hsp60 cassete was designed with a promoter and a terminator sequences derived from *Aspergillus nidulans*, a 20 bps protospacer and a scaffold region containing flanking ribozyme sequences. Guide RNA spacer was chose looking for the best localized PAM site and after BLAST demonstrate enough homology with the target. The Cas9 cassete was designed with the endonuclease sequence preceded by the nuclear localization signal SV40NLS, the hygromycin phosphotransferase gene and the Translational Elongation Factor promoter and terminator. The cassetes were inserted between different cloning sites to allow adaptations in the vector. Double digestion using the respective restriction enzymes, after transformation in TOP10 and Neb5 alpha cells, confirmed the insert sizes and the right localization. The next step will be to apply the CRISPR/Cas9-mediated gene editing to *Histoplasma* co-cultivating with *A. tumefaciens* LBA 4404 cells electroporated with the plasmid vector, to determine if the mutant fungal cells have changes on target gene mRNA expression and make tests *in vitro* and *in vivo* to verify the hsp60 absence impact in the virulence and pathology. To find new target determinants of *H. capsulatum* and attempts to modulate the host immunology response against the fungal are being pursued by researchers. After to verify the gene targeting disruption efficiency, another sequences can be cloned in this plasmid vector for new screenings.

Keywords: CRISPR/Cas9, *Histoplasma capsulatum*, histoplasmosis, gene edition

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