TITLE: EVALUATION OF *PEP4* GENE DELETION IN *CRYPTOCOCCUS NEOFORMANS*: STUDIES ON THE IMPACTS ON VIRULENCE FACTORS.

AUTHORS: FELIZARDO, G.[1,2]; PASCON, R. C.[2]; VALLIM, M. A.[2]

INSTITUTIONS: 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO – SP (Av. Prof. Almeida Prado, 1280 - Butantã, São Paulo – SP. CEP: 05508-900) – BRAZIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, CAMPUS DIADEMA, SÃO PAULO – SP, BRAZIL.

ABSTRACT:

Cryptococcosis is a systemic mycosis of great clinical importance which mainly affects immune suppressed patients. Currently, two distinct species stand out as human pathogens, *Cryptococcus neoformans* and *C. gattii*. This species, with worldwide distribution is responsible for high rates of morbidity and mortality. The treatment is limited to few drugs and some resistance to them has been reported elsewhere. Therefore, new research must be carried out to broad the knowledge regarding this yeast biology aiming at traits that could be new targets for antifungal drugs. Previously we showed that autophagy is important for virulence in *C. neoformans.* Therefore, the goal of this project is to expand the knowledge on the autophagy process in this pathogenic yeast. The mechanism of autophagy is a conserved process, responsible for the cell survival in the face of adverse nutritional conditions and environmental stresses. Here we evaluate the impact that lack of Pep4 (Protease A) has on important virulence traits of *C. neoformans,* such as high temperature (37°C); production of phospholipase; melanin; urease and polysaccharide capsule, as well as the response of yeast cell to oxidative (H_2O_2) and nitrosative ($NaNO_2$) stresses. In *S. cerevisiae* Pep4 is responsible for maturation and activation of vacuolar hydrolases within the autophagic vesicle. We deleted the *PEP4* gene from *C neoformans* genome by replacing the coding region with a cassette bearing the hygromycin resistance maker. The mutant phenotype was accessed on $NaNO_2$ and H_2O_2 supplemented medium (yeast extract, peptone and dextrose). Incubation was carried out at 30ºC and 37ºC for 48 hours. Capsule production was induced on CO_2-independent medium and cells were stained with India ink, and observed under light microscope. Also, the gene expression pattern associated with thermal, nitrosative stress and nutritional deprivation was evaluated. Total-RNA was extract with Trizol and subject to first strand synthesis, then subject to qPCR using SYBR Green. Our results shows that the *pep4* mutant of *C. neoformans* produces significantly more capsule than the wild type strain (KN99α) at 30°C and 37°C. Moreover, the *PEP4* gene expression is modulated by the combination of absence of carbon source, temperature and $NaNO_2$. *C. neoformans* Pep4 protein is important for the yeast cell survival after exposure to sodium nitrite suggesting that, this protein is involved in recycling damage proteins after nitrosative stress.

Keywords: *Cryptococcus neoformans,* Autophagy, *PEP4*, Virulence factors

Development agency: FAPESP 2015/04400-9, 2016/14542-8