TITLE: THE ROLE OF *HSP12* GENES IN STRESS RESPONSE AND VIRULENCE OF *CRYPTOCOCCUS GATTII.*

AUTHORS: MOTTA, H.; REUWSAAT, J.C.V.; CAMARGO, M.S.; VAINSTEIN, M.H.; STAATS, C.C.; KMETZSCH, L.

INSTITUTION: UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL, PORTO ALEGRE, RS (AVENIDA BENTO GONÇALVES, 9500 – SETOR 4 – PRÉDIO 43421 – LAB 222, CEP 91501-970, PORTO ALEGRE – RS, BRAZIL)

ABSTRACT:

The establishment of fungal infection caused by *Cryptococcus* cells relies on the expression of important determinants of pathogenicity, which include the ability to grow at host temperature (37 °C), the production of melanin on the cell surface, and the assembly of an immunomodulatory polysaccharide capsule associated with the cell wall. Despite the pivotal role of virulence determinants, the ability to overcome the stress response developed by the host's defences is also fundamental. For this, important changes occur in the gene expression profile of pathogenic yeasts. Heat shock proteins (HSP) have been shown to have fundamental activity in the viability of fungi in the host environment, as they modulate determinants of pathogenicity and the host's immune response. In this context, we characterize two Hsp12 proteins (*HSP12.1* and *HSP12.2* genes) in *C. gattii*. We demonstrated that both *HSP12* gene expression are increased during heat shock condition (30 °C to 39 °C). Through the construction of knockout mutants *hsp12.1, hsp12.2,* and a double mutant *hsp12.1/12.2*, we conclude that only the absence of Hsp12.1 resulted in a notable phenotype alteration. The *hsp12.1* mutant showed increase sensitivity to the H_2O_2 stressor, and *hsp12.1* and *hsp12.1/12.2* mutants have intracellular accumulation of ROS. Despite the accumulation of ROS, *hsp12.1* and *hsp12.1/12.2* mutant cells are resistant to paraquat, a reactive species inductor. The null mutant for Hsp12.1 also shows an SDS-sensitive phenotype probably by a destabilization of plasma membrane, suggesting that the Hsp12.1 acts as a chaperone in the plasma membrane as its orthologues in other fungi. From the perspective of host-pathogen interaction, the inactivation of *HSP12.1* leads to an increase in capsule size and phagocytosis rate by murine macrophages, and a hypovirulence phenotype in *Galleria mellonella*, an invertebrate systemic infection model. Thus, these results contribute to a better understanding of *C. gattii*-host interaction and demonstrate the importance of the *HSP12.1* gene for cryptococcal adaptation to stress during infection.

KEYWORDS: *Cryptococcus gattii*, Hsp12, Heat shock protein, virulence.