

Title: EVALUATION OF THE SENSIBILITY TO HYDROGEN PEROXIDE OXIDATION OF THE OOMYCETE *Pythium insidiosum*

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Abstract:

Introduction: Pythiosis is a chronic pyogranulomatous and life-threatening disease caused by the fungus-like organism *Pythium insidiosum*. The disease has been reported in tropical and subtropical areas, progresses rapidly, and if not treated in the early stages the infected host can die within weeks. In order to colonize a host and to start an infection, *P. insidiosum* need to adhere to the host tissue and need to avoid phagocytosis by neutrophils and macrophages. These cells use reactive oxygen species (ROS) as part of their arsenal to neutralize microorganisms. ROS can cause damage to biological macromolecules such as proteins and lipids. Unsaturated fatty acid chains of membrane lipids and, to a lesser extent, saturated lipids can react with ROS and undergo peroxidation. Reactions of ROS with proteins result in the oxidation of aminoacid side chains, often leading to a loss of function. Therefore, the aim of this study is to investigate *P. insidiosum* sensibility to hydrogen peroxide oxidation. **Material and methods:** 10 *P. insidiosum* strains isolated from equine pythiosis, identified by PCR-based assay, were used to perform this study. Zoospore inoculum was obtained by the zoosporogenesis technique and counted using a Neubauer chamber. The inoculum was diluted on distilled water to obtain a final concentration of 1×10^4 zoospores/mL. To evaluate the Oomycete sensibility to H₂O₂, we prepared Mueller-Hinton Agar plates with different concentrations of H₂O₂, which were 0,1; 0,25; 0,5; 1,0; 2,5; 5,0; 7,5 and 10,0 mM. 20 µL of the inoculum were put in the center of the plate and the diameter of the growth was measured after 24, 48, 72 and 96 hours. **Results:** The maximum resistance of *P. insidiosum* isolates to H₂O₂ was 2,5 mM, 4 isolates grown until 1 mM and 1 isolate grown until 0,5 mM. At the H₂O₂ concentration of 1,0 and 2,5 mM the growth ratio was lesser than on the lower concentrations. **Conclusion:** *Pythium insidiosum* has different sensibility to hydrogen peroxide oxidation between isolates, being is sensible to low concentrations of this oxidative compound.

Key words: Hydrogen peroxide; *Pythium insidiosum*; Pythiosis; Virulence factors.

Acknowledgements: CNPq, CAPES, FAPERGS.