

## EVALUATION OF THE ENZYMATIC ACTIVITY OF TRICHODERMA KONINGIOPSIS (POS7) AS POSSIBLE BIOCONTROLLER

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## **RESUMO**

INTRODUCTION: Plant pathogens cause diseases that result in a significant loss of quality and yield of valuable crops around the world. The use of specific soil mycolytic microorganisms to control plant pathogens is an ecological approach to overcome the problems caused by chemical pesticides used in plant protection methods. Many researchers recommend Trichoderma species as the preferred option for controlling phytopathogens. Trichoderma acts as biocontrol agent through the direct penetration of phytopathogenic fungi and/or secreting antifungal compounds, such as hydrolytic enzymes, mainly chitinases, glucanases and proteases that are capable of hydrolyzing the components of the cell walls of their host. Objective: Due to its important role in the biocontrol activity, the present study was carried out to evaluate and select carbon and nitrogen sources for the induction of the secretion of mycolytic enzymes, in the *Trichoderma koningiopsis* POS7 native strain of Misiones. METHODS: Microorganism: T. koningiopsis POS7 strain isolated from native agricultural soils of Misiones, Argentina. This strain is deposited in the fungal culture collection of the Institute of Molecular Biotechnology of Misiones, and previously was identified and selected by our working group. Screening was carried out in liquid culture medium. As carbon sources colloidal chitin 0.18 % (w/v); gelatin 5 % (w/v); cell walls treated of Fusarium sp. 2 % (w/v) and cell walls of Fusarium sp. untreated 2 % (v/v) were used. As nitrogen sources yeast extract, urea, ammonium sulfate and the Mandels nitrogen complex were used. The ability of T. koningiopsis POS7 to secrete mycolytic enzymes was quantitatively determined. To quantify protease activity, the azocasein method was used. For the  $\beta$ -1,3-glucanase and chitinase activities, the dinitrosalicylic acid method was used. The experiments were performed in duplicate on a four-level factorial design. Data were analyzed using Statgraphics Centurion XVI.I software. Tukey's test was used to evaluate and select variables that had a significant effect on enzyme activity. An analysis of variance (ANOVA) and a test of difference between means were performed, with a confidence level of 95.0 %. RESULTS: With respect to carbon sources, for the three enzymes studied the highest enzymatic activity with statistical differences (p <0.05) was observed in the media containing cell walls treated of Fusarium sp. Regarding to the nitrogen sources, for the three enzymes studied the highest enzymatic activity with statistical differences (p <0.05) was observed in the media containing yeast extract. Combining both factors, we observed an outstanding

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enzymatic activity of chitinases,  $\beta$ -1,3 glucanases and proteases, obtaining 63.50 U/L, 990.30 U/L and 398.22 mg/L respectively. With the other carbon sources tested, enzymatic activity was also observed but to a lesser extent CONCLUSION: Screening carried out in liquid culture medium allowed us to select the variables that showed statistically significant effects on the enzymatic secretion of chitinases,  $\beta$ -1,3 glucanases and proteases of *T. koningiopsis* POS7. In further works, each of these factors will be optimized for the secretion of enzymes involved in biological control using *Trichoderma*.