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SELECTION OF CARBON AND NITROGEN SOURCES FOR ENHANCING THE SECRETION OF MYCOLYTIC ENZYMES OF ESCOVOPSIS IN SUBMERGED FERMENTATION

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RESUMO

Introduction: In the province of Misiones (Argentina), one of the major pests affecting the forestry sector are leaf-cutter ants of the genera *Atta* and *Acromyrmex*. Their forage activity consists of cutting and transporting to nest fresh plant material, on which the fungus *Leucoagaricus gongylophorus* (Basidiomycota: Agaricales) grows, which is their main source of food. One the other hand, fungi of the genus *Escovopsis* (Ascomycota: Hipocreales) are considered specialist parasites of *L. gongylophorus*. These mycoparasites secrete extracellular mycolytic enzymes, mainly chitinases, glucanases, and proteases, which are capable of hydrolyzing the cell walls components of their host. Therefore, *Escovopsis* is a potential indirect biocontrol agent of leaf-cutter ants, and an effective way for its biotechnological application is to induce its mycolytic activity. Objective: In this study, the objective was to select carbon and nitrogen sources, which promote the greater secretion of mycolytic enzymes in a strain of *Escovopsis* native from Misiones. Methods: We used a promising strain of *Escovopsis*, previously identified and selected by our working group. Assays in submerged fermentation were performed. A factorial design of two and four levels, using different carbon sources (cell walls of *Fusarium* sp. and *L. gongylophorus*) and nitrogen sources (yeast extract, urea, ammonium sulfate and the Mandels nitrogenous complex) were performed. The *Escovopsis* capacity to secrete mycolytic enzymes was quantitatively assayed. Proteolytic activity though the azocasein method, and β-1,3-glucanase and chitinase activities by the dinitrosalicylic acid method were assayed. All the tests were done in duplicate and data were processed though the statistical software InfoStat 2018. To evaluate and select variables that had a significant effect on enzyme activity Tukey's test was used. Analysis of variance (ANOVA) and a test of difference between means were performed, with confidence level of 95.0%. Results: Regarding to carbon sources, a significant effect for the three analyzed enzymes ($p < 0.05$) was observed; and the media containing cell walls of *L. gongylophorus* showed the highest activity. Regarding the nitrogen sources, the highest enzymatic activity in the media that containing the Mandels nitrogenous complex was observed, with a significant effect for β-1,3-glucanases and chitinases ($p < 0.05$). Furthermore, the media containing urea and ammonium sulfate did not show activity for any of the analyzed enzymes. Particularly, for chitinases an interaction between the two studied factors was observed; and the tests that contained jointly, cell walls of *L. gongylophorus* and Mandels

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showed a significantly higher activity than the other assays ($p < 0.05$). Conclusion: The factorial design used allowed us to select the variables that presented statistically significant effects on the enzymatic secretion of *Escovopsis*. It was determined that enzymatic secretion is promoted when liquid fermentation is carried out using the cell walls of *L. gongylophorus* as a carbon source and the Mandels complex as a nitrogen source. Subsequently, each of these factors will be optimized.

PALAVRAS-CHAVE: β-1, 3-Glucanases, Biocontrol, Mycoparasite, Proteases, Chitinases

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