

STUDY BIOLOGICAL CONTROL ACTIVITY OF *TRICHODERMA HARZIANUM* BY ISOTOPE RATIO MASS SPECTROMETRY (IRMS)

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The study of the interactions among the microorganisms, especially between pathogen and other microorganisms, is very useful to identify possible biocontrol agents. The use of microorganisms labeled with stable isotopes could represent an efficient approach to study the direct parasitization or metabolites assimilation by microorganisms. A microorganism labeled with stable isotope can be monitored in the environment and, if it is directly parasitized or its metabolites are used by other microorganisms, the label can be detected using isotope ratio mass spectrometry (IRMS). In this study we isolated and identified 159 different species of fungi and bacteria naturally present in the soil and their ability in the control of a plant pathogen (*Armillaria mellea*) activity was investigated. The screening was carried out basing on the antagonistic and mycoparasitic activity of the isolates against the pathogen. The selection was carried out in vitro by dual-culture test, carried out in Petri plates containing labelled pathogen on the opposite side of *Trichoderma* and by IRMS analysis using ^{13}C isotope, to detect the active degradation and metabolic assimilation of the labeled *A. mellea* by the microorganisms. *Trichoderma harzianum* in contact with labeled *A. mellea* had higher $\delta^{13}\text{C}$ values ($244.03 \pm 36.70\text{‰}$) and was able to inhibit the pathogen development for $80 \pm 0.19\%$. This variation is able to explain an active degradation of the pathogen by *Trichoderma*. The microorganism is able to suppress *A. mellea* after 29 days in direct contact in a broth of malt extract. Studying the parasitism activity of the fungus in microcosm soil condition, *Trichoderma* present a mycoparasitism activity and its $\delta^{13}\text{C}$ values increase ($1.97 \pm 2.24\text{‰}$) after one month in direct contact with labeled pathogen. The microcosms were composed by 20 g of natural autoclaved soil, inoculated with pathogen-microorganism and stored under six different conditions, at 5 or 20% soil humidity and at 2, 10 or 20°C.