

TITLE: BIOCONTROL OF *Aspergillus carbonarius* AND OCHRATOXIN A IN GRAPE BERRIES USING *Bacillus* STRAINS

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ABSTRACT:

The fungal manifestation in grapes during their cultivation is quite common and *Aspergillus carbonarius* is one of the main responsible for the occurrence of Ochratoxin A (OTA) in this fruit. The International Agency for Research on Cancer (IARC) classifies OTA as possibly carcinogenic to humans (group 2B, year 1993). For this reason, in the conventional production of grapes, synthetic fungicides are applied in large quantities. The use of these pesticides is related to the presence of undesirable residues in the soil, water, and in grapes. In addition, they present toxicological risk for animals and are related to the development of Parkinson's disease in humans. In order to reduce these repercussions, biocontrol has been studied, for example the use of *Bacillus* species. *in vitro* tests demonstrated that four *Bacillus* strains from the Amazon region were promising in reducing the growth of *A. carbonarius* and the synthesis of OTA. The aim of this study was to evaluate the effectiveness of these strains of *Bacillus* in grape cultivars as a biofungicide alternative. For the experiment, Chardonnay cultivar was chosen. Grape berries were separated in two groups, wounded and unwounded berries. In the first group a 0,8 mm diameter wound was performed using a sterile needle in each of one of the berries. The assay was conducted with both groups as follows: grapes inoculated only with *A. carbonarius* for positive control (C1), only with distilled water for negative control (C2), and with cell suspension from each one of the four *Bacillus* strains (10^9 CFU/ml) concomitantly with spore suspension of *A. carbonarius* (10^3 spores/ml). Grape berries were kept in an incubator at 25°C for 7 days. Inhibition of fungal growth was assessed by visual inspection and colony counts after serial dilution in Dicloran Rose Bengal (DRBC) medium, and OTA extraction from the samples was performed with Liquid Chromatography Coupled to Mass Spectrometry (LC/MS-MS). The best results obtained were the inhibition of both fungal growth and OTA synthesis in the berries inoculated with *Bacillus* P1 strain. The mycotoxin was identified according to its accurate mass in the protonated ion $[M+H]^+$ form and fragmentation pattern. In sample C1, a concentration of 3200 µg/kg of OTA ($[M+H]^+$ 404.0650) was found, while in the sample containing *Bacillus* P1 strain OTA levels were not detected. The *Bacillus* strains used could be a viable option for the biocontrol of this toxigenic fungus in grape berries.

Keywords: *Bacillus*, *Aspergillus carbonarius*, biocontrol, grapes

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