Title: BIOCONTROL ACTIVITY OF *Bacillus* STRAINS FROM BRAZILIAN AMAZON BASIN AGAINST AFLATOXIN B1 PRODUCER *Aspergillus flavus*

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Abstract:

Aspergillus species are known to produce a number of mycotoxins, including aflatoxins. These toxins present hepatotoxic, teratogenic and carcinogenic effects on human and animal health. Biological control has been studied as alternative method to inhibit fungal growth and mycotoxin synthesis. Bacillus strains isolated from aquatic environment of Brazilian Amazon region have shown antifungal activity against filamentous fungi. There is no specific report of these isolates against growth of toxigenic Aspergillus and aflatoxin B1 (AFB1) production. The antifungal activity of these Bacillus isolates was studied through antibiosis assays, spore germination test and AFB1 formation. Iturin production by these bacteria was also investigated. Bacillus strains, obtained from the collection center at Laboratory of Applied Biochemistry and Microbiology (ICTA/ UFRGS, Brazil), were cultured in tryptone soy broth for 48 h at 37°C. Sterile molten potato dextrose agar (PDA) containing spore suspension of AFB1 producer A. flavus was transferred to Petri dishes. After solidification, each bacteria was inoculated on PDA plates. The inhibition zones were measured after 7 days of incubation at 25°C. To evaluate the effect on radial growth, Bacillus strains were added on PDA and fungal spore suspension was placed in the centre of each plates and incubated at 25°C for 10 days. The growing radius of the fungal colonies was measured and radial growth rate was calculated. For spore germination test, known amounts of the fungal spore and bacterial suspension were added in Yeast Extract Sucrose broth (YES) at 25°C for 48 h. Germinated spores were observed in optic microscope and the relative germination inhibition was calculated. The influence of bacterial strain on AFB1 production was carried out by growing both in YES broth at 25°C for 10 days. AFB1 was extracted with chloroform and determined by thin layer chromatography. Iturin from cell-free supernatants of bacteria with best antagonism results was extracted using n-butanol and analyzed by high performance liquid chromatography. All bacteria showed antifungal activity with inhibition zones ranging from 3 to 9 mm. Bacillus sp. P11 had better results with reduction on colony growth, spore germination and AFB1 production of A. flavus (84.2, 97.8 and 96.6%, respectively), besides the iturin production (237 mg/L). The genus Bacillus isolated from the Amazon region could be promising biocontrol candidate against toxigenic A. flavus.

Key words: Biocontrol, Bacillus species, iturin, mycotoxins, aflatoxin B1

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