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Detection of aflatoxigenic filamentous fungi by PCR

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INTRODUCTION

In the present study, the polymerase chain reaction (PCR) was optimized for using as a molecular technique to identify the aflatoxigenic filamentous fungi. The PCR was evaluated using the specific pair of primers for beta-tubulin amplification and the primers for *AFLR* gene related to aflatoxin biosynthesis

MATERIAL and METHODS

Microorganisms

The aflatoxigenic filamentous fungi: *Aspergillus flavus* and *Aspergillus parasiticus;* were isolated from Brazilian nuts that were harvested at Amazonia.

DNA extraction and PCR

The template for PCR was obtained using the Lee et al. (2004) method. PCR runs were carried out using the comercial kit PCR SuperMix (Invitrogen). The primers for beta-tubulin were: 5'-GGTAACCAAATAGGTGCCGCT-3'/ 5'-TAGGTCTGGTTCTTGCTCTGGATG-3'; and the primers for *AFLR* gene were: 5'-CGCGCTCCCAGTCCCCTTGATT-3'/ 5'-CTTGTTCCCCGAGATGACCA-3', that generate amplicons with 1300 and 630 bp, respectively. The PCR set up was: 1 initial cycle (5min/95°C); 40 cycles (30s/95°C – 30s/63°C – 60s/72°C) and a final cycle (4min/72°C).

RESULTS and DISCUSSION

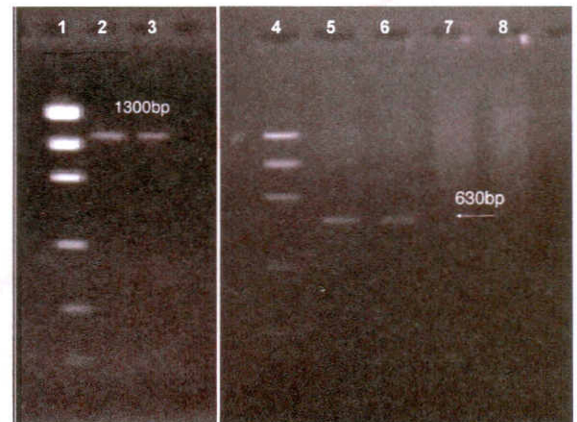

Figure 1: Agarose gel eletrophoresis of PCR runs using beta-tubulin and *AFLR* primers to detect aflatoxigenic filamentous fungi. 1 and 4-Low Mass Leader (Invitrogen); 2- beta-tubulin amplicon in *Aspergillus flavus*; 3- beta-tubulin amplicon in *Aspergillus parasiticus*; 5 and 6 – *AFLR* amplicon in *Aspergillus flavus*; 6 and 7- *Aspergillus parasiticus* DNA template without *AFLR* amplicon.

The results presented at Figure 1 confirmed the aplicability of Lee et al.(2004) method for a inhibitors-free DNA to performe PCR technique as a tool for detection of aflatoxigenic filamentous fungi. The absence of *AFLR* band in *Aspergillus parasiticus* sample suggests that its DNA has to be submitted to na additional purification step before PCR runs. This is due to the presence of pigments in the *A.parasiticus* extracted DNA, that can inhibit the PCR.

The continuation of the work will be related to carry out PCR runs using purified DNA from *A.parasiticus* and the use of primers for other genes of aflatoxin biosynthesis pathway.