MULTI-GENE PROBING OF AFLATOXIGENIC MOLDS IN FOOD USING PCR AND IMMUNOLOGICAL TECHNIQUES

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ABSTRACT

Aflatoxins are carcinogenic metabolites produced by several members of the *Aspergillus flavus* group in grains and foods. Three genes: *ver-1*, *omt-1* and *apa-2*, coding for key enzymes and a regulatory factor in aflatoxin biosynthesis respectively, have been identified and their DNA sequences published. In the present study, three primer pairs were generated, each complementing the coding portion of one of the genes. DNA extracted from mycelia of five *Aspergillus* species, four *Penicillium* species and two *Fusarium* species were used as PCR templates for each of the primer pairs. DNA extracted from peanut, corn and three insect species commonly found in stored grains was also tested. Positive results (DNA amplification) were achieved only with DNA of the aflatoxigenic molds *A. parasiticus* and *A. flavus* in all three primer pairs. The detection limit of the PCR was determined using the primer pairs complementing the *omt-1* and *ver-1* genes. Sterile corn flour was inoculated separately with six different molds, each at several spore concentrations. Positive results were only obtained following a 24-h incubation in enriched media, with extracts of corn inoculated with *A. parasiticus* or *A. flavus*, even at the lowest spore concentration applied (10^2 spores/g). No DNA amplification was observed from corn inoculated with other molds, even at the highest inoculum level (10^6 spores/g).

Polyclonal antibodies (pAb) were raised against an aflatoxigenic strain of *A. parasiticus* using two chimeric proteins, expressed in *Escherichia coli* as separate products, of the genes *ver-1* and *apa-2*. The gene products were amplified by PCR and each was cloned into the *E. coli* expression vector pGEX2T. Upon induction, the bacteria overexpressed a 38 kDa and 33 kDa chimeric proteins corresponding to the N-terminal domain of the genes *ver-1* and *apa-2*, respectively. The chimeric proteins were isolated and affinity-purified for use as antigens. The specificity of the raised antibodies was examined by ELISA. The polyclonal antibodies elicited against the chimeric proteins were highly specific, showing significantly higher ELISA absorbance values (A_{405}) against *A. parasiticus* and *A. flavus*, then against the other fungi tested and the corn grain. Such a "multi - antibody system" combined with the PCR technique, could provide a useful tool for the rapid, sensitive and accurate detection of aflatoxin producers present in grains and foods.