Abstract

Fungal infestation is a leading cause of qualitative and quantitative deterioration of stored wheat grains across the globe. Very limited information is available on the spatial distribution of fungal biota associated with stored wheat grains in India. In the present study, 284 pure fungal isolates were collected from stored wheat grains in the Food Corporation of India (FCI) warehouses located in three agro-climatic zones of West Bengal, India. All the isolates were classified into 29 OTUs based on amplified ribosomal DNA restriction analysis (ARDRA) of the ribosomal gene. The analysis of the internal transcribed spacer (ITS) sequence identified 24 fungal species within 29 OTUs and also showed that the maximum number of isolated fungi belongs to *Aspergillus flavus* (35%) of the family Ascomycota followed by *Rhizopus oryzae* (13%) and *Eurotium amstelodami* (9%). The nor-1 gene amplification analysis confirmed that 77% of these *A. flavus* isolates have the aflatoxin producing ability. Among different aflatoxins, the chemo type AFB1 is highly carcinogenic, and AFB1 was purified to homogeneity from a highly toxigenic *A. flavus* isolate by a series of chromatographic procedures. The anti-proliferative and apoptotic effect of this AFB1 on human hepatoma (HepG2) and embryonic kidney (HEK293) cells were studied, and IC_{50} was determined as 12.8 µM and 19.2 µM, respectively. In the AFB1 treated cells nuclear fragmentation, chromatin condensation and induction of apoptosis through the upregulation of the stress and apoptosis related genes like Bax, caspase-3 and cytochrome C, along with down regulation of anti-apoptosis gene, Bcl-2 were observed. Cell cycle analyses showed that AFB1 arrests cell growth in the sub G1 phase, and binds to DNA in a concentration dependent manner.

To develop a rapid, cost effective, fungal detection system in food grains, the fungal cell wall mannoprotein (MP1) gene of *A. flavus* was cloned, recombinantly expressed in *E. coli* and polyclonal antibody was raised against recombinant MP1 protein. Polyclonal antibody was also raised against inactivated spores of this fungus and purified. Using these antibodies presence of mycelia as low as 1 µg or 10^3 spores per gram of wheat grains were detected by enzyme linked immunosorbent assay (ELISA). The anti-MP1 antibody was found more sensitive than the anti-spore protein antibody and can detect Aspergillus as well as some other fungi. Western blot analysis showed the reactivity of these antibodies to several proteins present throughout the surface of mycelia and spores. For the detection of aflatoxin contamination in food grains, purified AFB1 was conjugated with BSA, and polyclonal anti-AFB1 antibody was raised in a rabbit and purified by antigen affinity chromatography. Purified anti-AFB1 antibody was conjugated with surface functionalized magnetic nanoparticles (MNPs) and used for the isolation of aflatoxin from the food grain sample. More than 80% of AFB1 was isolated from AFB1 spiked wheat grains and quantified through a sensitive colorimetric assay. The colorimetric assay was optimized to detect AFB1 contamination at the level of 2 µg/kg of wheat grains. These results suggest that the developed technique has the potential for both qualitative and quantitative analysis of aflatoxin present in food grains.

Keywords: Stored wheat grain, Fungal prevalence, *A. flavus*, Aflatoxin, Cytotoxicity, Apoptosis, Cloning of MP1, Antibody development, ELISA, MNPs, Colorimetric assay