Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

PRODUCTION OF AFLATOXIN BY *ASPERGILLUS FLAVUS* USING A MODIFIED MEDIUM AND TESTING ITS TOXICOGENIC ACTIVITY ON PLANT MODELS

Basil D'Mello[1*], Maithilee Raut[1], Rohan Gavankar[1], Deepa Verma[2] and Maya Chemburkar[3]
[1]Department of Biotechnology, VIVA College, Virar – West, India
[2]Department of Botany, VIVA College, Virar – West, India
[3]Department of Botany, Bhavan's College, Andheri – West, India

Received for publication: February 21, 2014; **Accepted:** March 14, 2014

Abstract: Aflatoxins are toxic, mutagenic, carcinogenic and teratogenic secondary metabolites posing health hazards to humans, animals and also adversely affect agricultural production. The medium was modified using freshly grated coconut as a substrate and was supplemented with 1 % Asparagine for Aflatoxin production using Aspergillus flavus. This modified medium proved to be an excellent source and yielded a maximum of 9 mg of Aflatoxin per gram of substrate used. The Broth filtrate was defatted with petroleum ether and Aflatoxin was extracted using soxhlet apparatus with methanol as the solvent. The Aflatoxin was detected using TLC. The effect of aflatoxin on seed germination and root elongation was tested on plant models -wheat and onion respectively.

Keywords: Aflatoxin, *Aspergillus flavus*, modified medium, toxic effect.

INTRODUCTION

Aflatoxins are mycotoxins with highly toxic and carcinogenic properties produced by some strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius.* These fungi are frequently found in foodstuffs and animal feeds. However, not all strains are able to produce aflatoxins, and this has encouraged the use of screening for their aflatoxin production abilities. Different strains of *Aspergillus* have been used for the production of aflatoxins on various substrates like Yeast extract-sucrose (YES) medium (1) and natural media with wheat, rice, or peanut (2).

Strains of *A. flavus* and *A. parasiticus* that produce aflatoxins are ubiquitous in nature. Aflatoxins have been frequently detected in grains, oil seeds, tree nuts, fermented beverages, milk, edible animal tissues and many other agricultural commodities and thus present a risk to human health that is insufficiently recognized (3,4,5).

The two major Aspergillus species that produce aflatoxins are *A. flavus* , which produces only B aflatoxins, and *A. parasiticus*, which produces both B and G aflatoxins. Aflatoxins M1 and M2 are oxidative metabolic products of aflatoxins B1 and B2 produced by animals following ingestion, and so appear in milk (both animal and human), urine and faeces. The main target organ for toxicity and carcinogenicity is the liver. It has an important role in the occurrence of some human diseases like hepatoma, gastritis, diarrhoea, fever, renal damage, hepatic disease, convulsions, etc (6). Aflatoxins used for experimental purposes are still obtained from laboratory cultures of *Aspergillus flavus.*

Both liquid and solid media have been used for microbiological preparation of it and yield largely depends upon the nature of substrate used (7). Testing large numbers of isolates for aflatoxin production on a variety of substrates is tedious and time-consuming. For this reason, several screening methods for direct visual determination of aflatoxin production have been developed. These methods use more or less complicated culture media containing additives to enhance the production of aflatoxins in order to achieve direct visual determination of a bright blue or blue-green fluorescent area surrounding colonies under UV radiation (8). It is not known whether if plants are susceptible to aflatoxin. In the work reported here, a novel medium was formulated for production of aflatoxin and its direct visualisation. Also it was investigated whether differences exist in susceptibility to aflatoxin in terms of Wheat seed germination and elongation of onion roots.

MATERIALS AND METHODS

Strain and Maintenance Medium

Aspergillus flavus MTCC 2798 was procured from IMTECH Chandigarh and was used for the production of Aflatoxin. The strain was revived and sub-cultured on Potato Dextrose Agar (PDA). The culture was maintained on PDA.

Inocula

The inoculum was prepared by suspending the spores of *A. flavus* in 0.1% Tween-80 solution. The spore suspension was inoculated in to the broth using a micropipette. 1 ml of the spore suspension was inoculated per 20g of substrate.

***Corresponding Author:**
Dr. Basil D'Mello,
Department of Biotechnology,
VIVA College, Virar – West, India

Media

Production medium-Modified Aflatoxin Producing Ability (APA) broth (9) was used as the production medium. 20 g of fresh grated coconut and 1% asparagine were added in a flask and the volume was made upto 100ml. The pH was adjusted to 4.5. The medium was autoclaved and inoculated with the spores. The production temperature was selected to be 28 °C as it is considered ideal for the production of aflatoxins in liquid medium [10]

Plate Screening test medium [Coconut Extract Agar (CEA)]

50 g of grated coconut was homogenized with 100ml of hot distilled water. The homogenate was filtered and the pH of the filtrate was adjusted to pH 7 with NaOH. Agar was added (20 g/liter), and the mixture was heated to boiling and cooled to about 50°C. The mixture was then autoclaved, cooled to about 40 to 45°C, and poured while being stirred into sterile petridishes (11).

Extraction of Aflatoxin:

The filtrate was defatted with petroleum ether in soxhlet apparatus. The extract was then further purified using soxhlet apparatus with methanol as the solvent. The purified aflatoxin was weighed.

Aflatoxin detection Chromatographic assay

The cultured modified APA was filtered and then filtrate was mixed with half the volume of chloroform. The tubes were vortexed and spun at 3000 rpm for 5 minutes. The organic phase was separated and was dried on a heating block at 65°C. The extract was analyzed by TLC using 80% Benzene: 15% Methanol: 5% Acetic Acid as the solvent system. The visualization of bands was carried out under UV light at 365 nm.

Plate screening test for Aflatoxin

The CEA plates were inoculated with *A. flavus* and after 4 day incubation the plates were visualized under UV for fluorescence.

Effect of Aflatoxin on seed germination in wheat

The wheat seeds were soaked overnight in water and these soaked seeds were put into petri plates with tissue paper. In one set of petri plates water was added and in second set aflatoxin solution in water was added. The effect was seen over a period of 5 days.

Effect of Aflatoxin on root elongation of Onion

Onion roots were allowed to grow in the presence and absence of Aflatoxin and the effect of the same on root elongation were studied.

RESULTS

After the first day, yellowish pigmentation was observed in the medium. Media Clumps were seen adhering to the walls of the flask. The Aflatoxin was defatted using petroleum ether and was purified using soxhlet with methanol as the solvent. After purification, the yield of Aflatoxins was found to be 8mg per gram of substrate used. The yield was found to be higher than the ones reported by other researchers. The increase in the aflatoxins yield can be because of the presence of asparagine.

Plate screening assay

After incubation the coconut agar plates were observed under UV light. Greenish colored fluorescence was observed. The greenish-blue colored fluorescence can be because of the presence of aflatoxins diffused into the agar.

Extraction of Aflatoxin

The Aflatoxin was defatted using petroleum ether and was purified using soxhlet with methanol as the solvent. After the separation and evaporation of the solvent, light yellow precipitate was observed. After purification, the yield of Aflatoxins was found to be 8mg per gram of substrate used. The yield was found to be higher than the ones reported by other researchers. The increase in the aflatoxins yield can be because of the presence of asparagine.

TLC

After the extract was spotted on to the Silica gel TLC plate, the plate was developed using the developing solvent and was visualized under UV light. Greenish colored bands were observed, confirming the presence of aflatoxins.

Figure 1: TLC of aflatoxin

Effect of Aflatoxin on seed germination in wheat

The wheat seeds in petriplates with water germinated after 24 hours of incubation whereas there was no germination in plates in which aflatoxin solution was added even after 5 days.

Effect of Aflatoxin on root elongation of Onion

It was observed that in onion roots growing in absence of the Aflatoxin showed root elongation of 20 mm but the roots allowed to grow in presence of aflatoxin grew only 5mm.

Treated

Control

DISCUSSION

Fresh grated Coconut proved to be a superior substrate for the production of Aflatoxin production. The yield of the mycotoxins increased to a greater extent due to the incorporation of Asparagine when compared to literature. The increase in yield can be because of the nature and content of neutral fats in the coconut kernel. *A. flavus* has lipolytic activities and so the glycerol formed due to the hydrolysis of fats would enhance growth and aflatoxins production.

Coconut-based media are known to be efficient for growth and aflatoxin production by *A. flavus* and *A. parasiticus*. The Coconut Agar medium is definitely advantageous as a plating medium in the context of the present investigation. This medium is opaque, and the whiteness of its surface is useful as a backdrop for distinguishing subtle differences in spore coloration and for observing early onset and intensity of sporulation among colonies. The Coconut Extract Agar surface is also highly absorptive for UV light and, as such, provides an effective background for detecting zones of fluorescent aflatoxins surrounding mini colonies. Detection by this plating method is technically analogous to examining coated thin layer chromatography plates under UV light for fluorescent aflatoxin spots.

The effect of Aflatoxin on plant models suggests that it inhibits the growth of roots and seedlings to a considerable extent. The potential of aflatoxin in inhibiting seed germination can also be related to its activity in inactivating DNA. In addition to the embryo, the seed contains a reservoir of preformed, essential molecules to sustain the developing embryo and seedling until it becomes nutritionally self-sufficient. Antimetabolites, such as aflatoxin, interfere with the synthesis rather than the utilization of metabolites and can only exert their toxicity when biosynthesis becomes essential to growth after the supply of preformed metabolites has been exhausted. When this occurs, continued growth of the seedling ceases or becomes dose dependent if the toxicant functions competitively by binding to an essential molecule such as DNA.

ACKNOWLEDGEMENT

The authors would like to thank the Principal and the Management of VIVA College of Arts, Commerce and Science for their support throughout the period of research work.

REFERENCES

1. Davis ND, Diener UL, and Eldridge DW. Production of aflatoxins B1 and G1 by *Aspergillus flavus* in a semisynthetic medium. Appl. Microbiol, 1966, 14, 378–380.

2. Hara S, Fennell DL, and Hesseltine CW. Aflatoxin producing strains of *Aspergillus flavus* detected by fluorescence of agar medium under ultraviolet light. Appl. Microbiol, 1974, 27, 1118–1123.

3. Abdulkadar HW, Al-Ali and Al-jedah J., Afatoxin contamination in edible nuts imported in Qatar. *Fd. Contr*, 2000, 11, 157-160.

4. Jaime-Garcia R and Cotty PJ, Spatial distribution of *Aspergillus flavus* and its toxigenic strains on commercial cottonseed from south Texas and its relationship to aflatoxin contamination. *Pl. Pathol*, 2006, 55, 358–366.

5. Qazi JI and Fayyaz Z, Aflatoxin contaminated foods and health risk perspective for Pakistani population. *Mycopath*, 2006, 4, 27-34.

6. Peraica M *et al.,* Toxic effects of mycotoxins in humans, Bulletin of the World Health Organization, 1999, 77 (9).

7. Abbas *et al.,* Cultural Methods for Aflatoxin Detection, Journal of Toxicology, TOXIN REVIEWS, 2004, 23, 2 & 3, 295-315.

8. Fente CA, Jaimez Ordaz, Va´Zquez BI, Franco CM, And Cepeda A., New Additive For Culture Media For Rapid Identification Of Aflatoxin-Producing *Aspergillus* Strains., Applied And Environmental Microbiology, 2001, 4858–4862.

9. Cutuli MT, Cuellar A, Camara JM, Mateos A, Suarez G. Different media and methodologies for the detection of aflatoxin production by Aspergillus flavus strains isolated from trout feed. Mycopathologia. 1991, 113(2), 121-125.

10. O'Brian GR, Georgianna DR, Wilkinson JR, Yu J , Abbas HK, Bhatnagar D, *et al.*, The effect of elevated temperature on gene transcription and aflatoxin biosynthesis, Mycologia ,2007, 99 (2) 232-239.

11. Davis ND, Iyer SK, and Diener UL, Improved Method of Screening for Aflatoxin with a Coconut Agar Medium, Applied and Environmental Microbiology, 1987, 1593-1595.

12. Arseculeratne SN, De Silva LN, Wijesundera S and Bandhunatha CHSR, Coconut as a medium for experimental production of aflatoxins, Applied Microbiology, 1969, 88-89.

Source of support: Nil
Conflict of interest: None Declared