Archive of SID

SCREENING OF AFLATOXIN-PRODUCING MOULD ISOLATES BASED ON FLUORESCENCE PRODUCTION ON A SPECIFIC MEDIUM UNDER ULTRAVIOLET LIGHT

M. Razzaghi abyaneh[1]*., A. Allameh*[2] *and M. Shams*[1]

Departments of Mycology[1] and Biochemistry[2], Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran

Abstract - *Aflatoxin contamination of food and feedstuffs is a serious economic and health hazard. Over a period of two years, 35 samples of raw pistachio nuts collected from different parts of Iran were analysed for the presence of aflatoxigenic strains of the Aspergillus flavus group. Eighty-five isolates of A.flavus and A.parasiticus were identified based on specific yellow-orange pigment production in the reverse of the colonies on Aspergillus flavus and parasiticus agar (AFPA) medium and microscopic criteria. These isolates were present in 65.71% of the samples and their aflatoxin-producing ability was examined. At first, 12 out of the 15 aflatoxin-producing isolates were selected based on bright blue fluorescence colour on aflatoxin-producing ability (APA) medium. Later, all of the isolates were identified after culturing on sucrose-low salts (SLS) medium. Furthermore, aflatoxin production on APA and SLS media was confirmed when the mycelia and media were extracted and analysed for aflatoxins on TLC plates. A.flavus strains produced mainly aflatoxin* B_1 *with concentrations ranging from 0.21 to 1.49 µg/g of APA medium, where as A.parasiticus strain produced all four aflatoxins (*B_1, B_2, G_1 *and* G_2*) with 3.71 µg aflatoxin* B_1*/g of the medium. Isolates other than A.flavus and A.parasiticus grown on this specific culture medium failed to produce a characteristic fluorescence under UV light. Evidences from this study indicate that fluorescent production on APA medium is a reliable screening test for distinguishing potential aflatoxin-producing strains of A.flavus and A.parasiticus from nontoxigenic strains.*

Acta Medica Iranica 38 (2): 67-73 ; 2000

Key Words: Aflatoxin, fluorescence, pistachio nut, Aspergillus, specific medium

INTRODUCTION

Aflatoxins are secondary metabolites produced by certain strains of the common moulds Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius and Aspergillus tamarii (1-3). These toxins with polyketide origin (Fig. 1) have been reported to be highly toxic, teratogenic, carcinogenic and mutagenic agents, which occur naturally in a wide variety of feed and feedstuffs (4,5). Under favorable conditions, toxigenic fungi grow on various agricultural commodities and produce mycotoxins e.g. aflatoxins (6,7) Outberaks of aflatoxin poisoning in human (liver cancer) and animals (decreased weight gain, hemorrhaging and suppression of the immune system) are closely related to consumption of foods and feeds contaminated with aflatoxin-producing strains (8).

Different authors have reported a number of screening methods for the detection of aflatoxin-producing moulds and most of them have used various media, large cultures and long incubation periods, which can involve considerable time, work and expense (9). These methods can be mainly divided in to three groups. The first group includes standard methods which are based on toxin production on both liquid or solid substrates, followed by purification procedures and final detection by thin-layer chromatography or high performance liquid chromatography (10, 11). In the second group, a small agar plug from a pure mould culture is used directly to apply the sample onto TLC-plate (12). Although this method is simple, it has disadvantages such as scattering of fungal spores during the operation. The third group includes methods for the detection of fluorescence from aflatoxins in liquid culture or an agar plate under the ultraviolet (UV) light (13-18). Generally, classic analytical methods are expensive and need sophisticated equipments and take long period of time for setup. However, screening of the fungi directly on a culture medium is simple and relatively fast procedure.

This study was undertaken to develop a method based on the formation of bright blue fluorescence of the agar medium surrounding colonies under UV light that would allow the rapid, reliable and unexpensive detection of aflatoxigenic isolates among a diverse groups of fungi. In order to screen potential aflatoxin-producing fungi on Iranian pistachio samples naturally contaminated with aflatoxin B_1, we have prepared a specific culture medium known as APA.

www.SID.ir

Archive of SID

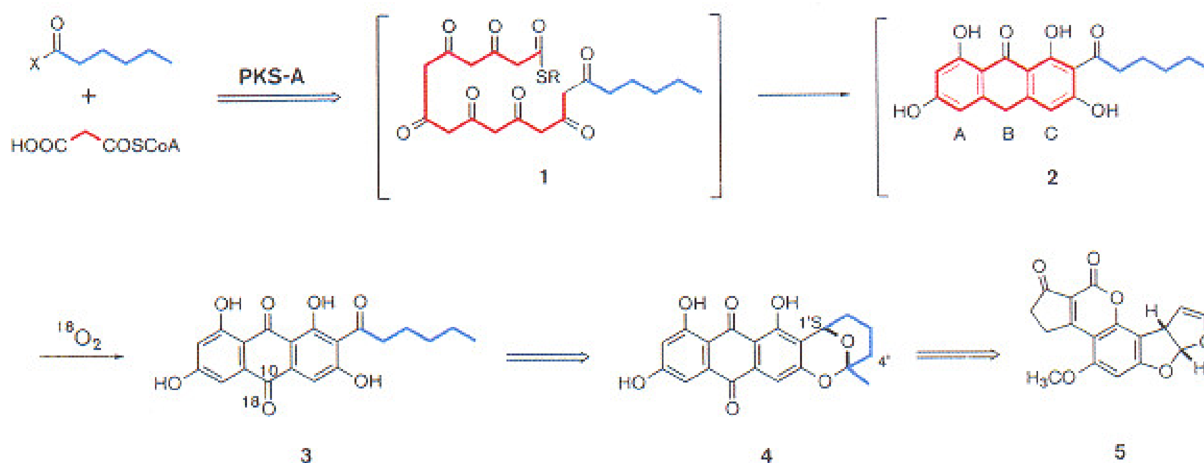

Fig. 1. Polyketide origin of aflatoxin biosynthesis. It is perposed that fatty acid synthase (FAS) enzymes synthesize the hexanoyl starter unit (in blue) to which a polyketide synthase (PKS) homologates malonyl units (in red) to initiate anthrone formation and aflatoxin biosynthesis (4)

1; primary intermediate, 2; anthrone, 3; norsolorinic acid, 4; averufin, 5; aflatoxin B_1

This medium is made up of ingredients which give blue fluorescence with aflatoxins secreted in the surrounding medium.

MATERIALS AND METHODS

Microorganisms

A total of 84 isolates of A.flavus Link and one isolate of A.parasiticus speare together with other isolates listed in Table 1 were cultured and analysed for their aflatoxin-producing ability. All of the isolates were isolated from samples of pistachio nuts determined to be contaminated with aflatoxin B_1. Likewise, A. parasiticus NRRL 2999, aflatoxin producer and A. flavus NRRL 3537 an aflatoxin-nonproducing strain were grown and extracted for aflatoxins as reference control strains.

Media

Primary isolation of fungal genera and species from pistachio nuts was done using three different common media including; Dichloran-rose bengal chloramphenicol agar (DRCA), Czapek-dox agar (CDA) and Sabouraud dextrose agar (SDA).

One-hundred seeds of each sample were taken, superficially sterilized with a solution of 0.4% sodium hypochlorite for 2 minute and subsequently washed three times with sterile distilled water. These seeds were then placed under aseptic conditions in mentioned culture media (8 seeds for each petridish) and incubated at 28 °C for 3 to 7 days.

A selective medium as Aspergillus flavus and parasiticus agar (AFPA) was used for rapid detection of A.flavus and A.parasiticus among the members of the genus Aspergillus (19). These species could produce a persistent bright yellow-orange pigment, near Cadmium Yellow under nonsporulating colonies (Fig. 2) The composition of the medium is given in Table 2. The isolates were cultured on AFPA in plates and incubated for 42-48 hour at 30 °C. Specific pigment is produced as a result of the reaction of aspergillic acid or neoaspergillic acid from the isolates with ferric ammonium citrate of the medium. Final identification of A.flavus and A.parasiticus as species among the Aspergilli were done according to the macroscopic and microscopic criteria (20).

Aflatoxin-producing ability (APA) medium was used in screening the mould isolates for their aflatoxigenicity (21). The composition of the medium is summarized in Table 3. To assay the aflatoxin production, five microliter of spore suspension (approximately 500 conidia) was inoculated at the center of APA medium in a glass petridish and incubated at 28 °C in the dark for 7 to 10 days. Plastic dishes are not suggested because of their interference with fluorescence production. Isolates of A.flavus and A.parasiticus were also screened for their aflatoxin-producing ability by using the semimicro culture technique with some modifications (22). Cultures were inoculated in 10 ml of sucrose-low salts (SLS) medium on 50 ml erlenmeyer flasks and incubated for 8 days at 28 °C before the extraction process.

Extraction and assay of aflatoxins

Total content of APA medium (about 20 g) showing blue fluorescence was removed and mixed with 50 ml of distilled water and then homogenized 3 times each time for about 2 minute in a Heidolph DIAX 600 homogenizer. The aqueous slurry was extracted for 10

www.SID.ir

Archive of SID

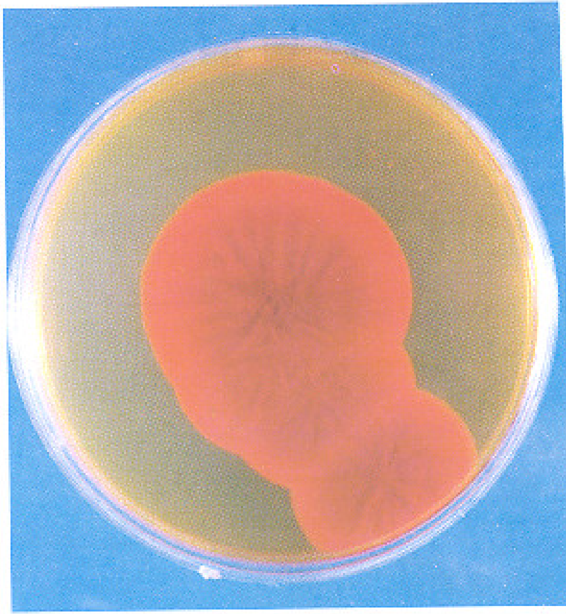

Fig. 2. Intense yellow-orange reverse coloration in colonies of A.flavus and A.parasiticus on AFPA medium

minute by mixing with 20 ml of chloroform. The mixture was then centrifuged, and the chloroform layer (lower phase) was separated and retained. Following the incubation period required for aflatoxin biosynthesis on SLS medium, flasks were subjected to a very short heat treatment at a temperature of 121 °C to kill the mould. Aflatoxins were extracted from mycelia and media with hot chloroform (55 °C). The chloroform fractions obtained from each culture media were pooled, filtered and concentrated near to dryness using vaccum evaporator apparatus (Heidolph WB 2000). Residues were dissolved in a known volume of chloroform and analysed by TLC technique on 20 × 20 cm silica gel precoated aluminium sheets (E. MERCK). The plates were developed using two different solvent systems known as chloroform-methanol (98:2, vol/vol) or acetone-chloroform-water (12: 88:15, vol/vol/vol). Aflatoxin spots were observed under UV light and then, for quantitative analysis, TLC plates were scanned with a Desaga CD 60 fluorodensitometer by comparison of samples with different dilutions of aflatoxin B_1 reference standard run on the same plates. Visualization of the spots was performed by spraying the plates with 25% sulphuric acid.

RESULTS

A total of 35 Iranian pistachio nut samples determined to be contaminated with aflatoxin B_1 were examined for the presence of fungal genera and species specially members of the genus Aspergillus. Based on the macroscopic and microcopic criteria, a total of 166 isolates belonging to 8 genera of the fungi were isolated from nuts (Table 1). The frequency of fungal contamination of nuts with genera Aspergillus, Penicillium and Alternaria was greater than other isolates. Table 4 shows the results of the specific blue fluorescence production of selected isolates on APA medium under UV light as a preliminary screening assay of aflatoxin production. The results clearly showed that only 12 out of the 85 A.flavus and A. parasiticus strains were fluorescent or APA-positive (Fig. 3).

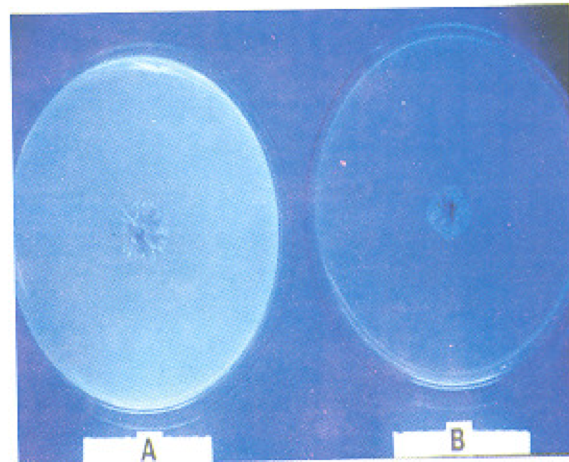

Fig. 3. Colonies of aflatoxin-positive (A) and aflatoxin-negative (B) strains of A.flavus and A.parasiticus on aflatoxin-producing ability (APA) medium under UV light showing characteristic fluorescence of agar surrounding the aflatoxin-producing strain.

These strains were designated as aflatoxin positive on APA medium after confirmation with complementary analysis. Aflatoxin production of these strains was directly observed on TLC plates (Fig. 4). The pattern of aflatoxin production on TLC plates by A.flavus and A.parasiticus strains was different. TLC chromatograms showed that four different naturally occuring aflatoxins known as B_1, B_2, G_1 and G_2 were produced by A.parasiticus strain, whereas toxigenic strains of A.flavus produced only B group of aflatoxins (Table 5). The presence of aflatoxin B_1 was confirmed by its R_f value which was comparable to aflatoxin B_1 standard (Fig. 4). The amount of aflatoxin B_1 produced by A.parasiticus strains on APA medium was highest among all of the isolated (3.45-3.71 μg/g medium). It is interesting to note that A.parasiticus isolated from pistachio (number 14) can produce even more aflatoxin B_1 than the standard strain (NRRL 2999) on APA medium. A nontoxigenic standard strain of A.flavus NRRL 3537 was grown and analysed for aflatoxin

www.SID.ir

Archive of SID

Table 1. Frequency of occurrence of principal fungal genera and species in pistachio nuts

Moulds	No. of isolates	No. of positive samples	Percent of contaminated samples
Aspergillus flavus	84	23	65.71
Aspergillus parasiticus	1	1	2.85
Aspergillus niger	15	8	22.85
Aspergillus tamarii	4	3	8.57
Aspergillus ochraceus	1	1	2.85
Aspergillus clavatus	1	1	2.85
Aspergillus spp.	2	2	5.71
Penicillium spp.	18	15	42.85
Fusarium spp.	8	5	14.28
Acremonium spp.	2	2	5.71
Cladosporium spp.	3	3	8.57
Alternaria spp.	12	9	25.71
Rhizopus spp.	9	8	22.85
Mucor spp.	6	5	14.28

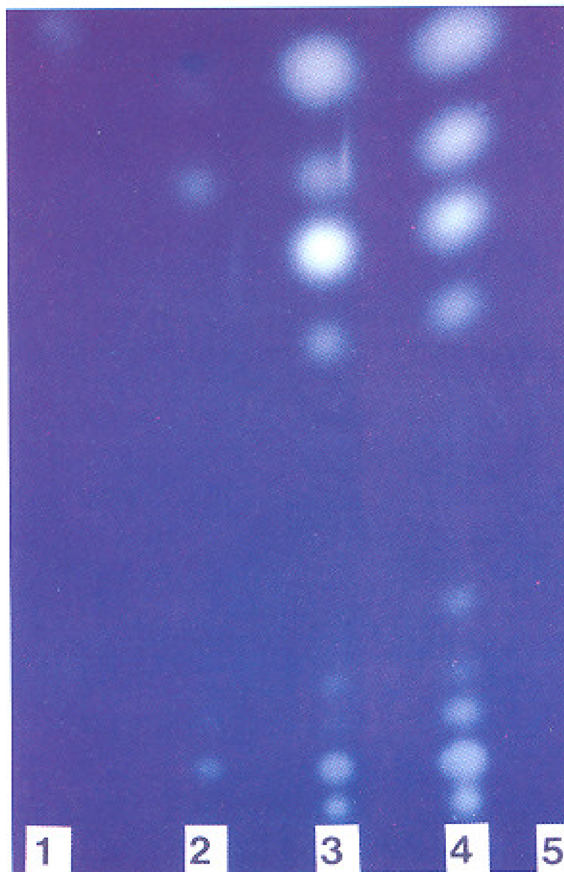

Fig. 4. TLC analysis of chloroform extracts prepared from the agar surrounding the mould colonies as described in Materials and Methods. The chloroform extracts developed by TLC and photographed by fluorescence. 1; aflatoxin B_1 (standard), 2; A.flavus 8, 3; A.parasiticus 14, 4; A.parasiticus NRRL 2999, 5; A.flavus NRRL 3537.

Table 2. The composition of Aspergillus flavus and parasiticus agar (AFPA) medium (19)

Peptone, bacteriological	10 g
Yeast extract	20 g
Ferric ammonium citrate	0.5 g
Chloramphenicol	100 mg
Agar	15 g
Water, distilled	1 litre
Dichloran	2 mg (0.2% in enthanol, 1.0 ml)

a. Initial pH of the medium was 6.2.

Table 3. The composition of aflatoxin-producing ability (APA) medium (21)

Distilled water	1 litre
$(NH_4)\ H_2PO_4$	10 g
K_2HPO_4	1 g
$MgSO_4.\ 7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4.\ 7H_2O$	0.01 g
Sucrose	30 g
$HgCl_2$	5×10^{-4} M
Corn steep liquor[b]	0.5 g
Agar	20 g

a. pH adjusted to 5.5 with 1N NaOH before the addition of agar.

b. prepared in our laboratory from fresh corn.

Table 4. Production of blue fluorescence on APA medium and assay of its correlation with TLC results

Moulds	No. of examined strains	No. of fluorescent strains	No. of aflatoxin producing strains	
			on APA	on SLS
Aspergillus flavus	84	11	11	14
Aspergillus parasiticus	1	1	1	1
Aspergillus niger	5	-	-	-
Aspergillus tamarii	4	-	-	-
Aspergillus ochraceus	1	-	-	-
Aspergillus clavatus	1	-	-	-
Aspergillus spp.	2	-	-	-
Penicillium spp.	4	-	-	-
Fusarium spp.	3	-	-	-
Alternaria spp.	4	-	-	-
Mucor spp.	2	-	-	-
Acremonium spp.	2	-	-	-
Rhizopus spp.	3	-	-	-
Cladosporium spp.	2	-	-	-
F.graminearum[a]	1	-	-	-
T. harizanum[b]	1	-	-	-

a,b: Known toxigenic strains

www.SID.ir

Archive of SID

Table 5- Production of aflatoxin by A.flavus and A.parasiticus isolates on APA and SLS media and assay of its concentration and fluorescent production on APA medium

Isolate number	Aspergillus species	Toxin identified on								Concentration of AFB_1 ($\mu g/g$ APA medium)	Fluorescence on APA
		APA				SLS					
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2		
2999	A.parasiticus NRRL	+	+	+	+	+	+	+	+	3.45	+
3537	A.flavus NRRL									-	-
14	A.parasiticus speare	+	+	+	+	+	+	+	+	3.71	+
59	A.flavus Link	+	+			+	+			1.49	+
35	A.flavus Link	+				+				1.24	+
24	A.flavus Link	+	+			+	+			1.16	+
7	A.flavus Link	+				+				1.12	+
13	A.flavus Link	+				+				0.86	+
16	A.flavus Link	+				+				0.68	+
75	A.flavus Link	+				+				0.57	+
19	A.flavus Link	+				+				0.51	+
66	A.flavus Link	+				+				0.42	+
2	A.flavus Link	+				+				0.37	+
8	A.flavus Link	+	+			+	+			0.21	+
43	A.flavus Link					+				-	-
31	A.flavus Link					+				-	-
48	A.flavus Link					+	+			-	-

APA : Aflatoxin-producing ability medium AF: Aflatoxin SLS : Sucrose-low salts medium

production and used as a negative control strain. This strain and all of the other aflatoxin-nonproducing strains isolated from pistachios did not produce any fluorescence on APA medium (Table 4). In order to further evaluate the UV test results, all of the APA negative strains of A.flavus were grown on SLS medium which supports aflatoxin biosynthesis and then analysed for aflatoxin production by TLC. The results showed that only 3 out of the 73 APA-negative strains gain the ability to produce aflatoxin (Table 5).

DISCUSSION

A wide range of the fungi are broadly distributed in the environment which have the capability to contaminate many agricultural commodities. For toxigenic fungi, this contamination leads to the occurrence of certain mycotoxins in final products. The increasing significance of mycotoxins as potential public health hazards has aroused interest in the examination of certain foods and feeds for the presence and incidence of moulds and their ability to produce mycotoxins. Aflatoxin contamination of food and feedstuffs particularly in a large consignment could be determined using several approaches.

Chromatographic procedures such as HPLC used for the final quantitation of aflatoxins are generally tedious, expensive and time consuming. In addition, they require careful sampling and solvent extraction steps perior to analysis. Isolation of aflatoxin-producing fungi from suspected foodstuffs and identification of their ability to produce aflatoxins using fast and reliable mycological methods under laboratory conditions could be used as a preliminary screening test perior to chemical analytical methods. One of the considerable advantages of these methods is that early exposure of foodstuffs with aflatoxigenic fungi could be easily derermined which indicate the predisposition of these materials to aflatoxin contamination.

In present study, the mycoflora of pistachio nuts with special reference on aflatoxigenic fungi were determined using general and specific culture media. At first, the Aspergilli were separated from other fungal genera. Later, strains of A.flavus and A.parasiticus were identified and then grown on a specific culture medium known as APA medium for evaluation of their aflatoxigenicity. Aflatoxin production was primarily noticed by the change in colour of the medium surrounding colonies as a bright blue fluorescence under UV light. Further experiments based on chromatographic analyses e.g. TLC were carried out to confirm aflatoxin-producing ability of the strains.

Various fungal genera and species were isolated from the samples of pistachio nuts as summarized in Table 1. In addition to the presence of toxigenic

www.SID.ir

Archive of SID

A.flavus strains, many other species belonging to the genera Penicillium and Fusarium isolated in this study could be potentially mycotoxigenic. It has been established that the isolates of A.flavus and A.parasiticus vary greatly in their ability to aflatoxin production (23-26). However, about one-third (30-40%) of the naturally occuring A.flavus strains isolated from agricultural commodities are usually aflatoxigenic (27). In the present study, 17.6% of the isolates were capable of producing aflatoxins out of which approximately 80% had been able to produce specific blue fluorescence on the APA medium under UV light. Under different conditions using other food products, investigators could identify between 30 to 67% of the aflatoxin-producing strains based on their blue fluorescence criteria on the APA medium (28). Fortunately, we did not come across with strains which give false positive results on the APA medium. However, others reported that some fungal metabolites such as asperopterins A or B, flavacol and deoxy-hydroxyaspergillic acid might contribute to blue fluorescence production (29, 30). Furthermore, examined strains other than A.flavus and A.parasiticus could not produce blue fluorescence on the APA medium as shown in Table 4.

These data clearly indicated the specificity of APA medium to identify only aflatoxin-producing strains among various toxigenic and nontoxigenic fungal genera and species. The possibility of converting low aflatoxin producers so called APA-negative to potential aflatoxin-producing strains on SLS medium which supports aflatoxin biosynthesis was also examined. Among 85 isolates studied, only 3 false negative isolates were identified.

Briefly, blue fluorescence production by aflatoxigenic strains on the APA medium is due to the ingredients known as corn steep liquor and $HgCl_2$ (21, 31, 32). These compounds may enhance the accumulation of aflatoxin precursors by blocking various enzyme systems (21). It is clear that assay of fluorescence production on APA medium can not facilitate the identification of every aflatoxin-producing isolates but undoubtedly, strong producers will always be discovered. Finally, it is concluded that the observation of blue fuorescence on APA medium provides a simple, reliable and relatively fast way of preliminary screening of a large number of moulds for their aflatoxin producing ability.

Acknowledgement

The authors are grateful to Mr. Sadeghi from Slide House of Iran for excellent photographs, to Mrs. Vazeeri for quantitative TLC analyses and to Mrs. Nagahi from Institute of standard and Industrial Research for pistachio samples.

REFERENCES

1. Payne, GA. process of contamination by aflatoxin-producing fungi and their impact on crops. In Mycotoxins in Agricultural and Food Safety. (KK. Sinha. D. Bhatnagar. Eds), pp. 279-306. Marcel Dekker Inc. New York. 1998.

2. Kurtzman, CP, Horn, BW and Hesseltine, CW. Aspergillus nomius. a new aflatoxin-producing species related to Aspergillus flavus and Aspergillus tamarii. Antonie Van Leeuwenhoek.. 53(3); 147, 1987.

3. Goto, T., Wicklow, DT and Ito, Y. Aflatoxin and Cyclopiazonic acid production by a sclerotium-producing Aspergillus tamarii Strain. Appl. Environ. Microbiol. 62: 4036, 1996.

4. Watanabe, C.MH, Willson, D, Linz, JE, and Townsend, CA. Demonstration of the catalytic roles and evidence for the physical association of type I fatty acid synthases and a ployketide synthase in the biosynthesis of aflatoxin B_1. Chemistry & Biology, 3; 463, 1996.

5.Minto, RE and Townsend CA. Enzymology and molecular biology of aflatoxin biosynthesis. Chemical Reviews. 97; 2537; 1997.

6. Wiseman, DW, and Marth, EH. Growth and aflatoxin production by Aspergillus parasiticus when in the presence of streptococcus lactis. Mycopathologia. 73: 49, 1981.

7. Frank, HK. Diffusion of aflatoxins in foodstuffs. J. Food. Sci. 33; 98, 1968.

8. Brown, MP, Brown - Jenco, CS and Payne, GA. Genetic and molecular analysis of aflatoxin biosynthesis. Fungal Genetics and Biology, 26; 81. 1999.

9. Diener, UL and Davis, ND In: Golblatt, LA (ed) Aflatoxins: Scientific background, control and implication. Academic, New York, pp. 13-45. 1969.

10. Association of Official Analytical Chemists. Official methods of analysis. P. 447. 14th ed. Association of Official Analytical Chemists. Arlington, Va, 1984.

11. Manabe, M, Goto, T and Matsuura, S. High-performance liquid chromatography of aflatoxins with fluorescence detection. Agrtic. Biol. Chem., 42; 2003, 1978.

12. Filtenborg, O and Frisvad, JC. A simple screening method for toxicogenic moulds in pure cultures. Lebensm. Wiss. Technol., 13; 128, 1980.

www.SID.ir

Archive of SID

13. Dyer, SK and Mc Cammon, S. Detection of toxigenic isolates of Aspergillus flavus and related species on coconut cream agar. J. Appl. Bacteriol. 76(1); 75, (1994).

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

15. Lee EG and Townsley, PM. Chemical induction of mutation or variation on aflatoxin producing cultures of Aspergillus flavus. J Food. Sci. 33; 420, 1968.

16. Bennett, JW and Goldblott, LA. The isolation of mutants of Aspergillus flavus and Aspergillus parasiticus with altered aflatoxin producing ability. Sabouraudia, 11; 235, 1973.

17. de Vogel, P, van Rhee, R and Koelensmid, WAAB. A rapid screening test for aflatoxin-synthesized aspergilli of the flavus-oryzae group. J. Bacteriol. 28; 213, 1965.

18. Davis, ND, Iyer, SK and Diener, UL. Improved method of screening for aflatoxin with a coconut agar medium. Appl. Environ. Microbiol. 54; 1593, 1987.

19. Pitt, JI, Hocking, AD and Glenn, DRJ. An improved medium for the detection of Aspergillus flavus and A.parasiticus. Appl. Bacteriol., 54; 109, 1983.

20. Raper, KB and Fennell, DJ. The genus Aspergillus, The Will and Wilk Co., Baltimore, pp. 259-272, 1965.

21. Hara, S, Fennell, DI and Hesseltine CW. Aflatoxin-producing strains of Aspergillus flavus detected by fluorescence of agar medium under ultraviolet light. Appl. Microbiol., 27; 1118, 1974.

22. Scott, PM., Lawrence, JW and Walbeek, W. Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts. Appl. Microbiol., 20; 839, 1970.

23. Lee, Y.W. and Kim, JG. A study on the isolation of aflatoxin-producing strains from agricultural products in korea. Asia. Pac. J. Public Health., 6(4); 179, 1993.

24. Abarca, ML, Bragulat, MR, Castella, G and Cabanes FJ. Mycoflora and aflatoxin-producing strains in animal mixed feeds. J. Food. Protec., 57(3); 256, 1994.

25. Jimenez, M, Mateo, R, Querol, A, Huerta, T and Hernandez, E. Mycotoxins and mycotoxigenic moulds in nuts and sunflower seeds for human consumption. Mycopathologia, 115; 121, 1991.

26. Lillard, HS, Hanlin, RT and Lillard, DA. Aflatoxigenic isolates of Aspergillus flavus from pecans Appl. Microbiol, 128, 1970.

27. Richard, JL, Bhatnagar, D, Peterson, S, and Sandro, G. Assessment of aflatoxin and cyclopiazonic acid production by Aspergillus flavus isolates from Hungary. Mycopathologia, 120; 183, 1992.

28. Cvetnic, Z and Pepelijnjak, S. Aflatoxin-producing potential of Aspergillus flavus and Aspergillus parasiticus isolated from samples of smoked-dried meat. Die Nahrung., 39; 302-307, 1995.

29. Kaneko, Y and Sanada, M. Studies on the fluorescent substances produced by Aspergillus fungi: VIII. Purification and isolation of asperopterin B and chemical properties of asperopterin B and A.J. Ferment. Technol, 47;8, 1969.

30. Yokotsuka, T, Sasaki, M, Kikuchi, I, Asao, Y and Nobuhara A. Studies on the compounds produced by moulds. Part 1. Fluorescent compounds produced by Japanese industrial moulds. Nippon Nogei Kagaka Zasshi, 41; 32, 1967.

31. Codner, RC, Sargeant, K and Yeo, R. Production of aflatoxin by the culture of strains of Aspergillus flavus-oryzae on sterilized peanuts. Biotechnol, Bioeng. 5; 185, 1963.

32. Schroeder, HW. Effect of corn steep liquor on mycelial growth and aflatoxin production in Aspergillus parasiticus. Appl. Microbiol, 14: 381, 1966.

www.SID.ir