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Molecular detection and identification of *Aspergillus fumigatus* in potato chips by PCR technique

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Abstract
Aspergillus infections have grown in importance in the last years , *A.fumigatus* is a member of the genus *Aspergillus*, it can colonize a wide variety of substrates , the chips that produced from potatos are one of these substrates that infected by this genus , so in this study we isolated *A.fumigatus* from potato chips (Lays)with three flavored(salt,French cheese,vinegar) according to colony character in petridish compare with PCR technique for certainity.
Keywords : *aspergillus fumigatus*,PCR technique, potato chips

Introduction

Potato chips are quick foods usually derived from one or more basic food items , and are eaten from children and adult , they can be processed from potato tubers (Alabi,2007 ; Okoruwa,1997) . Potato chips are designed to be less perishable and more durable it often contain preservatives and appealing ingredients such as peanuts and specially designed flavors(Street food,2006).

The chips prepared outside of home setting,in factories then transport to super market or shops , therefore there is need to certain its safety in terms of the presense, and the quantity of aflatoxigenic moulds and aflatoxins .Aflatoxins are mycotoxins produced by Aspergillus species they are known to be hepatotoxic ,carcinogenic and teratogenic .Hepatocellular carcinoma resulting from chronic aflatoxin exposure is well documented (IARC,2006) .

Aspergillus is a genus of mitosporic fungi , it is frequently implicated in life- threating infections in immune-compromised patients (Montero,2005) it is a large genus of filamentous fungi which characterized by a unique spore bearing structure (conidiophore) and reproduced by forming mitotic spores which made it one of the most common fungi on earth (Klich,2009).A. fumigatus is one of this genus which is known to cause infection in humans (85% of cases) , this study aimed to isolate A. fumigatus from potato chips according to fungal colony morphology , microscopy and molecular detection by PCR technique .

Materials and Method

1.1 Samples: a total of 12 potato chips (lays)with three flavored (salt, French cheese,vinegar)were purchased from shops in Hilla / Iraq . The samples comprised of potato and specific flavor of the three ,all samples were grouped into three based on flavor then transported to microbiology laboratory,Biology Department ,College of Science for Women ,Babylon University.

1.2 Isolation and characterization of *Aspergillus* :in this study we used PDA agar (20gm potato,20gm sucrose,15gm agar,1000 ml d.w.)then crashed potato chips made it powder and spreaded on agar plate ,incubated (28°-7 days)then purification on PDA agar.

1.3 DNA Extraction : we used for extracted DNA extract kit supplied from (Promega, Madison Wi,USA) which include : DNA Rehydration solution , Protein Precipitation solution ,Cell lysis solution ,Nuclei lysis solution ,RNase solution ,in addition EDTA from BDH-chem.LTD bool Company ,Lyticase enzyme from US biological co. , Isopropanol , Ethanol 70% .According to kit protocol DNA extracted of A.fumigatus then stored at 2-8°c until used in next step(Ciardo et al.,2010) .

1.4 PCR technique : we used specific solution which include:

1- T.E. buffer supplied from (Promega, Madison Wi,USA) .
2- Specific primer supplied from Bioneer Co./USA for detection *A.fumigatus* (Konstantinova et al,2002;) , (table 1)
3- Master mix supplied from Bioneer Co./USA ,include:
    a- Taq DNA Polymerase .
    b- (d NTPs)250 Mm from dTTP – dCTP – dGTP –dATP
    c- Tris-HCl (pH 9.0)
    d- KCl

In PCR step we used PCR apparatus (fig .1) by added specific primer with extract DNA into PCR tube which contain Master mix with total volume20 ml (table 2) according to master mix protocol.

PCR amplification condition were : 5 min initial step , followed by 38 cycles at 94°c for 1min ,59°c for 1 min and 72°c for 1.5 min and final extension step at 72°c for 75 min .after that amplification products were examined

by electrophoresis in agarose gel (1 gm agarose , 100 ml T.B.E buffer , stained with 2 ul Ethidium Bromide) according to (Sambrook et al.,1989) we used electrophoresis apparatus (fig.2) then the result visualized under U.V. light by U.V. transilluminator .

RESULT AND DISCUSSION

Microscopic examine revealed the unique spore bearing structure (conidiophore) for *Aspergillus* sp. to the 10 isolates which agree with (Klich,2009) in shape of this fungus structure (fig.3) .
And colony morphology for this 10 isolates was similar to this genus that agree with (Jankaan,2007) (fig.4) .
but depend on PCR technique using specific primer (fig. 5) we found that 5 of 10 isolates were *A. fumigatus* which is(1,5,7,9,10) similar to (Logotheti et al.,2008) results were they used specific primer tested in simplex PCR assays and were successfully amplified by PEX1-PEX2 primer pair producing amplicons of 200 bp.
While the other 5 was not *A. fumigatus* may be another species of *Aspergillus* , so we achieved that we can′t depend on morphology or microscopy in classify microorganisms with the development of biotechnology.

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Table 1. Specific primer supplied from Bioneer Co./USA

Aspergillus	**Primer pairs**	**Sequence(5′-3′)**	**Amplified region of aspergillus genome**	**Amplicon size(bp)**
A.fumigatus	PEX1 PEX2	TATGTCTTCCCCTGCTCC CTATGCCTGAGGGGCGAA	PEP aspergillopepsin 4 th axon	250 bp

Table 2. Total volume according to master mix protocol

solution	**volume**
Master mix	5 ul
DNA	5 ul
Forward primer	2.5 ul
Reverse primer	2.5 ul
Deionized water	Complete volume to 20 ul
Total	20 ul

Figure 1. PCR apparatus

Figure 2. Electrophoresis apparatus

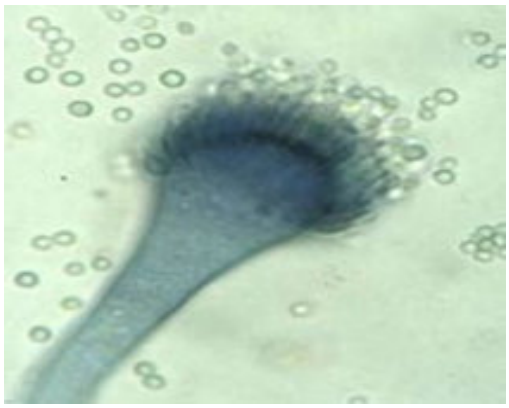

Figure 3. Conidiophore with conidia of *Aspergillus fumigatus*

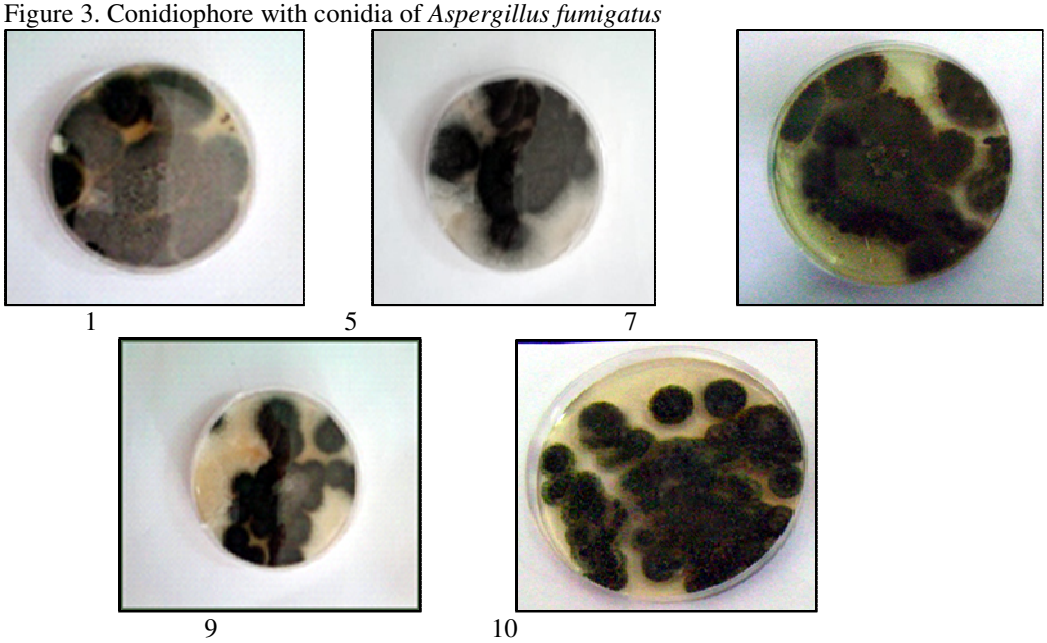

Figure 4. Colony shape of positive *Aspergillus fumigatus*

1 2 3 4 5 6 7 8 9 10

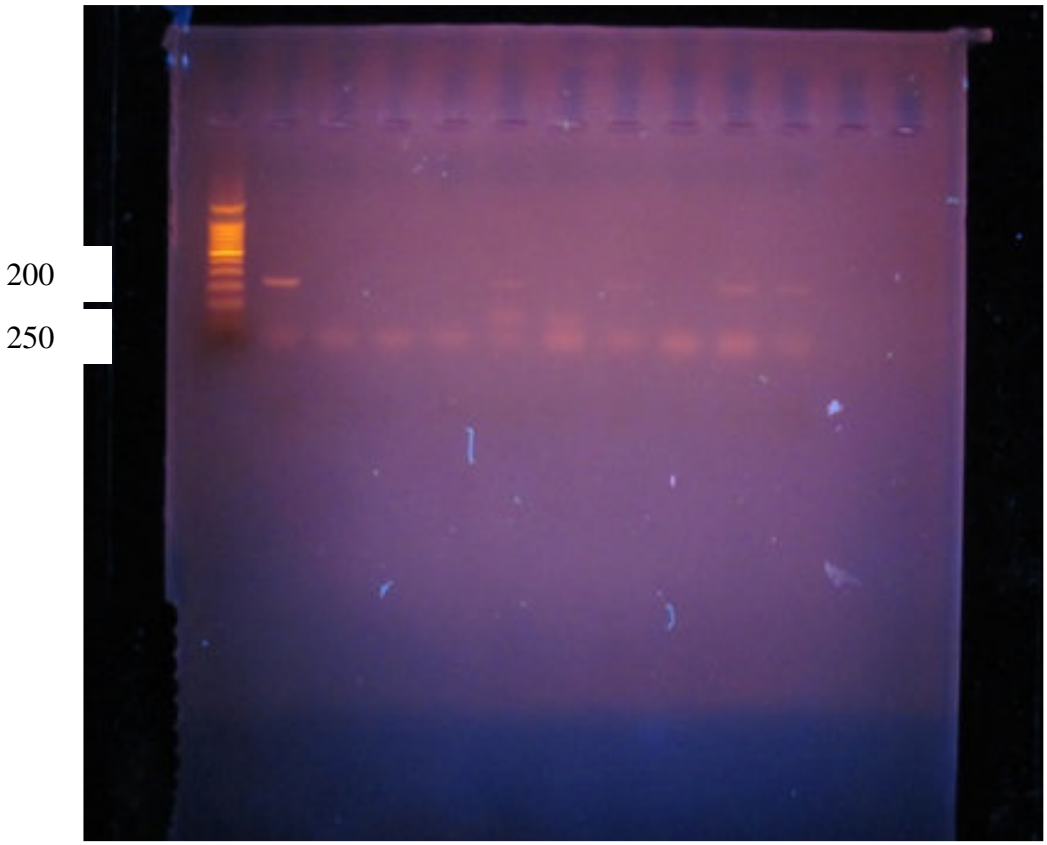

Fig .5 Electrophoresis gel visualized under U.V. light