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Study on fungal contamination of somechicken meat products withspecial reference to the use of PCR for its identification

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ABSTRACT

This work was carried out to evaluatethe fungalcontamination of chicken meat products sold in local markets as well as identification of some isolated moulds using PCR technique.Foridentification of the isolated moulds,samples were subjected to mycologicalexaminationusing themorphological (macroscopic and microscopic)characterization.Molecular identification using (ITS) was carried out for isolated*Aspergillus* and *Penicillium* species. The average total mould counts in the examined samples ofchickenluncheon, chicken pane and chicken minced meat were 3.1 x $10^2 \pm 0.82$ x 10^2, 7.4 x $10^2 \pm 5.4$ x 10^2 and 1.7 x $10^2 \pm 0.16$ x 10^2cfu/gm, respectively. In the examined samples, 9mould genera were identified. The identified mould genera were*Aspergillus, Eurotium,Penicillium,Geotrichum,Fusarium,Cladosporium*, *Mucor*, *Eupencillium* and *Acremonium* species. The isolated species of*Aspergillus parasiticus* and *Penicillium purpurogenum*were subjected to PCR identification, and were sequenced in bothdirections. Sequences were analysed and aligned by Clustal method using the program of DNAstar (Laser-gene, Wisconsin, USA).

Keywords: *A. parasiticus*, chicken meat, PCR, sequences,*P.purpurogenum.*

INTRODUCTION

Poultry meat industry started in Egypt in the mid 1960 with a competitive advantage over other meat industries. Meat is a perishable food item because it contains all the nutrients required for microorganisms to grow, and its pH (5.5–6.5) is not inhibitory to most microorganisms. The extensive fabrication, handling and distribution of raw and processed meat further increases exposure to microbial contamination. Some of the principal contamination sources encountered during processing are the slaughtering and evisceration processes**(Barbut, 2002).**Poultry meat productsmay be contaminated from raw

materials, workers, equipments, feathers, feet, faeces and skin if GMP (Good Manufacturing Practice) not applied**(Barbut, 2002).**In addition to processing procedures as scalding, evisceration, and cooling. However, mould and yeasts are of great importance in spoilage of poultry meat products resulting in different changes in flavor, color, texture and odor and also these fungi responsible for major portion of food deterioration especially in poor developing countries. This may be attributed to lake of hygienic measure and the use ofcontaminated additive and spices which considered a major important sources of mould contamination **(Abd El-Rahman, 1987).**

Polymerase chain reaction (PCR) is a technique widely used in fungal research. One of its advantages, is the ability to amplify very small amounts of DNA, in the picograms range, even in the presence of diverse contaminants. In spite of this, most of the extraction protocols of fungal DNA are designed for the obtaining of microgram amounts of highly purefied DNA, requiring the establishment of relatively large fungal cultures and long extraction procedures. These protocols are needlessly complicated for PCR experiments. On the other hand, some authors have pointed out the feasibility of using single spores (1) or boiled mycelium (2) as a source of DNA in PCR experiments. This is advantageous for detection purposes, but when working with hundreds of strains in population studies, obtaining the material from the culture plate can becumbersome and favor contaminations**Cenis (1992)**.

Therefore, the present study was planned out to throw a light on thetotal mould counts of chicken meat products (pane, minced meat and luncheon), as well as differentiation and species identification of contaminating fungi isolated from these products using PCR technique.

MATERIALS AND METHODS

Collection of samples

A total of hundred and eighty (180) samples of processed chicken products (60 samples of each chicken pane, chicken luncheon and chicken minced meat) were collected from shops and supermarkets. These samples were obtained and preserved in an ice box, until transferred to the laboratory under complete aseptic conditions examined as rapidly as possible.

Fungal isolation and identification

Total fungal count was carried out according to the techniques recommended by **ISO (217-1-2:2008)**.Fungi were isolated and identified according to macroscopical and microscopical characteristics as described by **Pitt and Hocking (2009)**.

DNA extraction and PCR amplification

Genomic DNA of the strains was obtained using the genomic DNA Extraction Kit (Gene JET Genomic DNA purification Kit Thermo scientific, Lithuania) following the manufacturer's instructions. DNA concentration was determined spectrophotometrically at 260/230 nm. The PCR primers used for identification of *Aspergillus* and *Penicillium* spp are listed in (Table 1). The PCR reaction was performed in an Gradient Thermal cycler (1000 S Thermal cycler Bio-RAD USA).The reaction mixture (total volume of 50 µl) was 25 µl Dream green PCR Mix (DreamTaq Green PCR Master Mix (2X) Fermentas Company, cat., No.K1080, USA.), 5 µl target DNA, 2 µl of each primers (containing 10 p mole/ µl) and the mixture was completed by sterile D. W. to 50 µl .

***Table (1)**: General primer used in PCR reactions for the identification of Aspergillus and Penicilliumspecies.*

Primer	Primer Design	Amplicon Size
Forward ITS1	5´- TCCGTAGGTGAACCTGCGG-3´.	550 bp
Reverse ITS4	5´- -TCCTCCGCTTTATTGATATG3´.	

PCR master Mix:

DreamTaq Green PCR Master Mix (2X) Fermentas Company, cat., No.K1080, USA.)

PCR amplification conditionsfor all strains was: 4 min initial step at 94°C followed by 35 cycles at 94 °C for 1 min, 56 °C for 1 min and 72°C for 1 min and a final extension step at 72 °C for 10 min. Amplification products were electrophoresed in agarose gels (1.5 % w/v) (Sigma, USA), which was used for running of DNA. Stained with Ethidium bromide using GeneRuler 100bp DNA Ladder: Fermentas Company, Cat.No.SM0243, US.

DNA fragment purification Kit: The amplified DNA fragments were purified using Gene JET PCR purification kit (USA) and were sequenced by Chromogen Company, Germany. The two strains were sequenced in both directions. Sequences were analysed and aligned by Clustal method using the program DNAstar (Laser-gene, Wisconsin, USA).

RESULTS AND DISCUSSION

Moulds only compete with bacteria on meat when storage temperatures are lowered to 0^0C or below, or when the meat surface dries to an a_w that enables fungi to compete.In earlier literature, spoilage of chilled or frozen meat by fungi was usually attributed to *Mucorales*, especially *Thamnidiumelegans*and *Mucor*species, which grew as ‘‘whiskers’’ on cold stored meat **Pitt and Hocking (2009).Michener and Elliott (1964)** cited several

reports on bacteria and fungi growing on meats at -5^0C, with yeasts and mouldspredominating as temperatures were further lowered, to a limit at about -12^0C.**Schmidt-Lorenz and Gutschmidt (1969)** reported that moulds and yeasts grew on chickens stored at – 7.5 and -10 ± 0.2^0C for 1 year. Spoilage of chilled meats in postwar years has principally been the result of ‘‘black spot’’, traditionally believed to be due to *Cladosporiumherbarum*.

The results achieved in figure (1) revealed that the incidence of mould in the examined chicken meat product samples were 40 (66.67%), 55 (91.7%) and 37 (61.67%) for chicken luncheon, chicken pane and chicken minced meat, respectively. The results obtained for chicken luncheon, chicken pane and chicken minced meat are similar to that recorded by many investigators such as**Shaltout (2002),Bkheet*et al.* (2007)**,and **Wadee (2010)** who mentioned that, about 86.6%of chicken luncheon as well as chicken minced meat samples have mould contamination. While the examined chicken pane samples revealed mould isolation with an incidence of 93.33%.From the economic point of view, mould and yeast lead to certain defects that may change the food quality or render it unfit for human consumption.

The previous results recorded in table (2) showed that the total mould count of the examined positive chicken luncheon,chicken pane and chicken minced meatranged from 20 to3×10^3 with a mean value of $3.1 \times 10^2 \pm 0.82 \times 10^2$,5 x 10 to 3.1×10^3 with a mean value of $7.4 \times 10^2 \pm 5.4 \times 10^2$ and <10 to 5.1×10^2 with a mean value of $1.7 \times 10^2 \pm 0.16 \times 10^2$ cfu/g, respectively. Higher figures were reported by **El-Gazzar (1995), Shaltout (1996), Farag (2000) and El-Deeb*et al.* (2011)** who reported that the total mould counts in examinedchicken luncheon, nuggets, and fillets were $7.5x10^3 \pm 2.4x10^3$, $7.8x10^3 \pm 0.3 x10^3$ and $7.8x10^3 \pm 0.2x10^3$cfu/g, respectively.

The obtained results revealed that the ready to eat meals as luncheon usually contaminated with moulds if the moisture content exceed 10% ,mould can grow on the surface and resist the fall in pH, giving a final pH value of 6.0-6.2. The comminution of poultry meat greatly increases thesurface area and distribution of the microorganisms throughout thecreating microenvironment **(Saad *et al.*, 1989).**While whole poultry carcasses tend to have a lower microbial count than cut up poultry ***(*Jay, 1978).**

Table (3) showed that theincidence ofthe moulds isolated from chicken luncheon, chicken pane and chicken minced meat samples was as the following: *A. niger* (10.0%), (13.3%) and (15.0%) respectively,*A. flavus* (13.3%), (8.3%) and (15.0%) respectively.*A. parasiticus*was isolated with an incidence of (1.7%) and (3.3%), from the examined chicken pane and chicken minced meat samples, respectively, *A. ochraceus*and*A. terreus*were isolated from chicken pane and chicken minced meat samples and its incidence was (3.3%) and (1.7%), (1.7%) and (3.3%) respectively, while*A. candidas*wasisolated fromthe examined chicken luncheon and chicken panewith an incidence of(1.7%) for each.*A. clavatus* isolated only from chicken minced meat samples (1.7%). While, the number and percentage of *Penicillium* species isolated from the examined chicken luncheon, chicken pane and chicken minced meat samples were 4 (6.7%), 6 (10 %) and 1(1.7%), respectively for *P. corylophilum*, while the number and percentage of identified *P. citreonigrum* were1(1.7%),2(3.3%) and 1(1.7%), respectively.

On the other hand such number and percent for the isolated*P. simplicissimum*, *P. purpurogenum and P. thomii* were 1(1.7%) and 2(3.3%), 2(3.3%) and 1(1.7%), 1(1.7%) and 2(3.3%) fromchicken luncheon, chicken pane and chicken minced meat samples respectively. Meanwhile, *P. griseofulvum* and *P. verrucosum* could be identified from only the examined chicken pane sample with number and percentage of 1(1.7%).

Also , results given in table (3) showed that *Geotrichum* species, *Fusarium* species ,*Mucor* species, *Eupencillium* species and *Acremonium* species could be isolated from10 (16.7%) , 2 (3.3%), 3 (5.0%),4 (6.7%) and 1(1.7%) of Chicken luncheon ,respectively. *Geotrichum* species, *Fusarium* species, *Cladosporium* species, *Mucor* species and *Eupencillium* species could be isolated from 3 (5.0%), 4(6.7%), 1(1.7%), 2 (3.3%) and 5 (8.3%) of examined chicken pane samples, respectively. *Geotrichum* species, *Mucor* species, *Eupencillium* species and *Acremonium* species could be isolated from 7(11.7%), 3(5.0%), 5 (8.3%), 3 (5.0%) and 4(6.7%), of examined chicken minced meat samples such mould genera could be isolated by **Shaltout (2002),Altalhi and Albashan, (2004) Hussein (2008) Hassan *et al.* (2012) and El-Diasty*et al.* (2013).**

Aspergillus flavus and *A. niger* caused lung disease when they grow and produce spores in the lungs. They were opportunistic and invade wounds, cornea and external ear in immuno-suppressed patients, it could cause pneumonia **Jacquelum (1999).***P.purpurogenum* considered as an important fungi as it secretes rubratoxins, a mycotoxins, which originally suggested as a main reason of mouldy corn toxicosis, or haemorrhagic anaemia in chickens **(Burnside *et al.*, 1957; Forgacs *et al.*, 1958 and Pitt and Hocking, 2009).***Penicilliumpurpurogenum* was isolated from cases of people with pneumonia, ear infections, keratitis, endocarditis, peritonitis, and urinary tract infections **(Johanning*et al*., 1999).**

Aspergillusparasiticus is one of the main sources of aflatoxins, the most important mycotoxins in the world's food supplies. Aflatoxins are produced in nature by *A. parasiticus, A. flavus* and a number of other species, including *A. nomius*, which are of little practical importance in foods **(Pitt and Hocking, 2009)**. The important differences in mycotoxins production between *A.parasiticus* and *A. flavus* are that *A. parasiticus* produces

G as well as B aflatoxins, while*A. parasiticus* isolates often produce aflatoxins in much higher concentrations **(Pitt, 1993)**also,non-toxigenic*A. parasiticus* strains are rare. Aflatoxins are both acutely and chronically toxic to both animals and human and may be responsible for greatly increasing susceptibility to many kinds of disease agents incountries where aflatoxin ingestion is common**(Wogan, 1992; Wang and Groopman, 1999; Williams *et al.*, 2004).**They have long been known to produce four distinct effects: acute liver damage, liver cirrhosis, induction of tumors and teratogenic effects **(Stoloff, 1977).** However more recent information indicates that the consequences of prolonged aflatoxin exposure are more widespread, including immune-suppression and interference with protein uptake **(Williams *et al*., 2004).**

Different concepts have been used by mycologist to define the fungal diversity; one of them is the morphological study, which is the classic approach where units are defined on the basis of morphological characteristics and ideally by the differences among them.This type of study is not sufficient for diversity study whereas the genetic diversity on the basis of molecular marker defeat differences among organism on the basis of size ofamplified DNA, which not influence by environmental factor. Variations (mutations) on nucleotides can't be studied by morphological markers while the molecular marker may overcome such type of problem.Therefore molecular marker reveal characterization is very effective for microbial species characterization.

. Twoof the isolated mouldsfrom chicken meat products were identified on morphological basis in present investigation (one isolate of *A. parasiticus*and one isolate of*P. purpurogenum)*were randomly selected for further confirmation via cloning and sequencing the ITS (Internal transcribed region) of the DNA. These regions (ITS) contain most conserved sequence at the terminal region and also contain the hypervariable

sequences distinguishing between species. Therefore, they have been considered as the best tool for the identification of the fungi. The use of ITS region as compared with other molecular probes is advantageous due to many reasons including increased sensitivity because of existence of more than 100 copies per genome **(Mirhadi*et al*., 2007).**

Conclusions

It can be concluded that chicken meat products are highly contaminated with various types of moulds as a result of spore concentration in poultry meat products as improper processing and negligence. Also, the data suggested that contamination may be due to inadequate refrigeration and absence of sanitation conditions which are the principal causes of higher levels of moulds contamination and increased species diversity. Poultry meat products especially ready to eat as luncheon, must be adequately fried before eating for at least 10 minutes at 80 ºC in home. Application of Food Safety Management System ISO 22000 with HACCP to poultry industry, particularly for poultry meat products should be applied to prevent or minimize all hazards including moulds, yeasts and mycotoxins.Molecular methods (PCR method), is a practical,the most sensitive, and least time-consuming method, as well as, it is considered as the most authentic way for microbial identification and have become the most common tool for the identification of fungi in food samples where genus *Aspergillus* and *Pencilliu*mare the most dominant mycotoxin producing strains isolated from poultry meat products in our studies.

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الملخص العربي

دراسة علي التلوث الفطري لبعض منتجات لحوم الدواجن مع الاشارة الي استخدام تفاعل البلمرة المتسلسل للتعرف علي الفطريات

استهدفتهذهالدراسة تقييممدي تلوث منتجات لحوم الدواجن المتداولة في الاسواق وتقييمالتلوث الفطريلكل من لانشون الدجاج و البانية ولحوم الدجاج المفروم وتصنيف الفطريات المسببةللأمراضوالفسادفيهذهالمنتجاتبأستخدامتفاعلالبلمرةالمتسلسل . كان متوسطالعدالكللفطرياتبالنسبة للانشون الدجاج و البانية ولحوم الدجاج المفرومهوعلى التوالى $3.1 \times 10^2 \pm 0.82 \times 10^2$ و $7.4 \times 10^2 \pm 5.4 \times 10^2$ و $1.7 \times 10^2 \pm 0.16 \times 10^2$

مستعمرة/ جرام علىالترتيب . تمعزلوتصنيف تسعةأنواعمنالعفن.الأنواعالتى تمعزلهامنالأعفان اشتملتعلىأجناسالأسبرجيليس، اورتيم،البنسيليوم، الجيوتركيم، الفيوزريم، الكلادسبوريوم، الميكور، ايوبنسيليوم و الاكريمونيم . تمالتعرفعلىبعضالعزولاتالممرضةوالمسببةللفسادبتلكالعيناتوهمنالاسبرجيليس والبنسيليوم بأستخدامتفاعلالبلمرةالمتسلسل . كانالتسلسل للاسبرجيليس بارازتكس والبنسيليوم بروبرجينم فيكلاالاتجاهين. تمتحليلالتسلسلعنطريقاستخدامبرنامج الحمضالنووينجمة (ليزرالجينات،ويسكونسن،الولاياتالمتحدةالأمريكيه)

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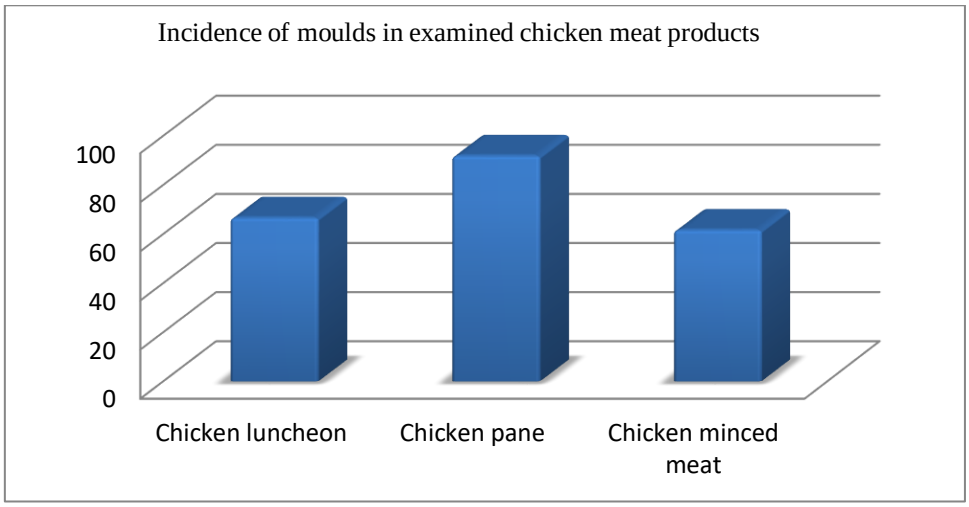

Figure (1): Incidence of moulds in examined chicken meat products

Table (1): Total mouldcounts (CFU/g) of chicken meat processed products:

Products	Min.	Max.	Mean ± SE.
Chicken luncheon	20	3×10^3	$3.1 \times 10^2 \pm 0.82 \times 10^2$
Chicken pane	50	3.1×10^3	$7.4 \times 10^2 \pm 5.4 \times 10^2$
Chicken minced meat	<10	5.1×10^2	$1.7 \times 10^2 \pm 0.16 \times 10^2$

The total number of examined sample for each product is 60 (N=60).

362 **Table (3): Incidence of identified mouldspecies in examined chickenmeat products:**

Mould genera	Chicken luncheon		Chicken pane		Chicken minced meat	
	No.	%	No.	%	No.	%
***Aspergillus* species**						
A. flavus	**8**	**13.3**	**5**	**8.3**	**9**	**15.0**
A. parasiticus	**0**	**0**	**1**	**1.7**	**2**	**3.3**
A.niger	**6**	**10.0**	**8**	**13.3**	**9**	**15.0**
A. ochraceus	**0**	**0**	**2**	**3.3**	**1**	**1.7**
A. terreus	**0**	**0**	**1**	**1.7**	**2**	**3.3**
A. clavatus	**0**	**0**	**0**	**0**	**1**	**1.7**
A .candidas	**1**	**1.7**	**1**	**1.7**	**0**	**0**
***Eurotium* species**						
E. chevalieri	**1**	**1.7**	**2**	**3.3**	**0**	**0**
E. repens	**0**	**0**	**1**	**1.7**	**0**	**0**
***Pencillium* species**						
P.corylophilum	**4**	**6.7**	**6**	**10.0**	**1**	**1.7**
P.griseofulvum	**0**	**0**	**1**	**1.7**	**0**	**0**
P.citreonigrum	**1**	**1.7**	**2**	**3.3**	**1**	**1.7**
P. brevicompactum	**0**	**0**	**0**	**0**	**1**	**1.7**
P.simplicissimum	**1**	**1.7**	**2**	**3.3**	**0**	**0**
P.purpurogenum	**0**	**0**	**2**	**3.3**	**1**	**1.7**
P.thomii	**2**	**3.3**	**1**	**1.7**	**0**	**0**
P. verrucosum	**0**	**0**	**1**	**1.7**	**0**	**0**
Geotrichum	**10**	**16.7**	**3**	**5.0**	**7**	**11.7**
species*Fusarium* species	**2**	**3.3**	**4**	**6.7**	**0**	**0**
Cladosporium species	**0**	**0**	**1**	**1.7**	**3**	**5.0**
Mucor species	**3**	**5.0**	**2**	**3.3**	**5**	**8.3**
Eupencillium species	**4**	**6.7**	**5**	**8.3**	**3**	**5.0**
Acremonium specie	**1**	**1.7**	**0**	**0**	**4**	**6.7**

363 The % was calculated according tothe total number of examined sample (N=60 for each

364 product)

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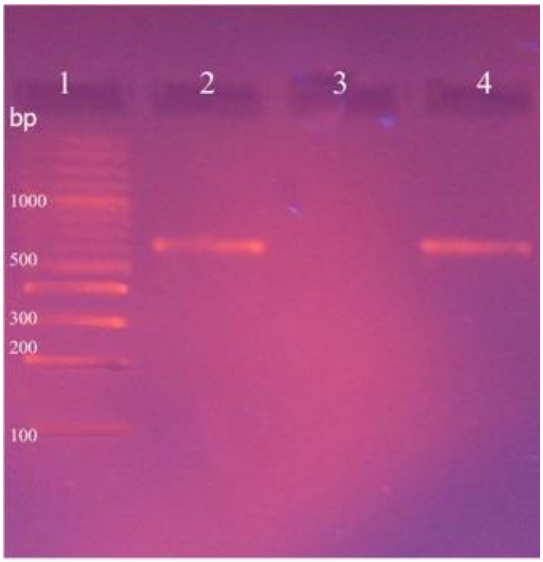

Photo (1): Agarose gel electrophoresis of*Aspergillus*spp. DNA (PCR)resulting from PCR amplification, single PCR performed with genomic DNA,Lane 1: 100bp DNA ladder, Lane 2: Control Positive, Lane3: Control Negative and Lane 4: sample

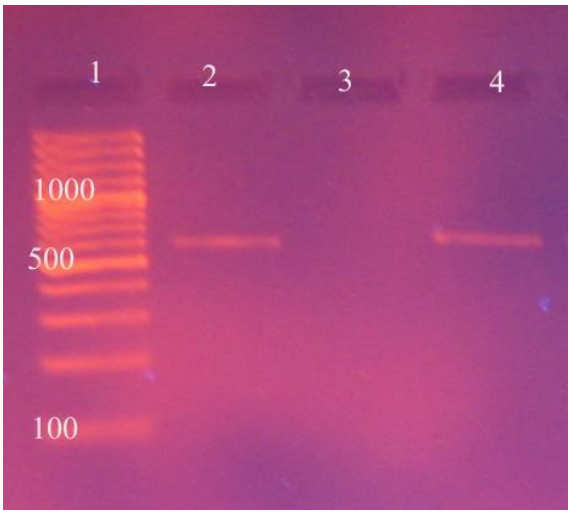

Photo (2): Agarose gel electrophoresis of*Penicillin* spp. DNA (PCR)resulting from PCR amplification, single PCR performed with genomic DNA,Lane 1: 100bp DNA ladder, Lane 2: Control Positive, Lane3: Control Negative and Lane 4: sample

Primer sequence of A.parasiticus and Penicilliumpurpurogenum

A.parasiticusForward primer sequence

GATCTCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCC
TGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGGGTTGGGCCGCCGTC
CCCTCTCCGGGGGGGGACGGGCCCCAAAGACAACGGCGANCCGCGTCCGATCCT
CGAGCGTATGGGATTTGTCACCCGCTCTGCCCCCCGGCCGGCGCTTGCCGAACG
CAAAACAACCATTTTTTCCAGGTGACCTCTCATCAGGTAGGGATACCCGTTGAA
TTTAACTATATCCTAATCGAAGCA

A.parasiticusReverse primer sequence

TGTTTTGCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCC
CATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCC
CCGGAGAGGGGACGACGACCCAACACACAAGCCGTGCTTGATGGGCAGCAAT
GACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCA
AAGACTCGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCT
GCGTTCTTCATCGATGCC

PenicilliumpurpurogenumForward primer sequence

GTCTTCTGAGTGCGAGACCCTCGCGGGTCCACCTCCCACCCGTGTCTCTTGAAT
ACCCTGTTGCTTTGGCGGGCCCACCGGGTCGCCCCGGTCGCCGGGGGGCACTG
CGCCCCCGGGCCTGCGCCCGCCAGAGCGCTCTGTGAACCCTAATGAAGATGGG
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CTAGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCGGGAGGGGCCTGCGGG
CGTTGGCCACCCACGATATTTTTTTACCGTTGACCTCGGATCAGGTAGGAGTTA
CCCGCTGAACTTAAGCATATCAAAAGTGGGGGAGAGAAAATTAT

PenicilliumpurpurogenumReverse primer sequence

AGATTTCGGGGTACTTCCTACCTGATCCGAGGTCAACGGTAAAAAAATATCGT
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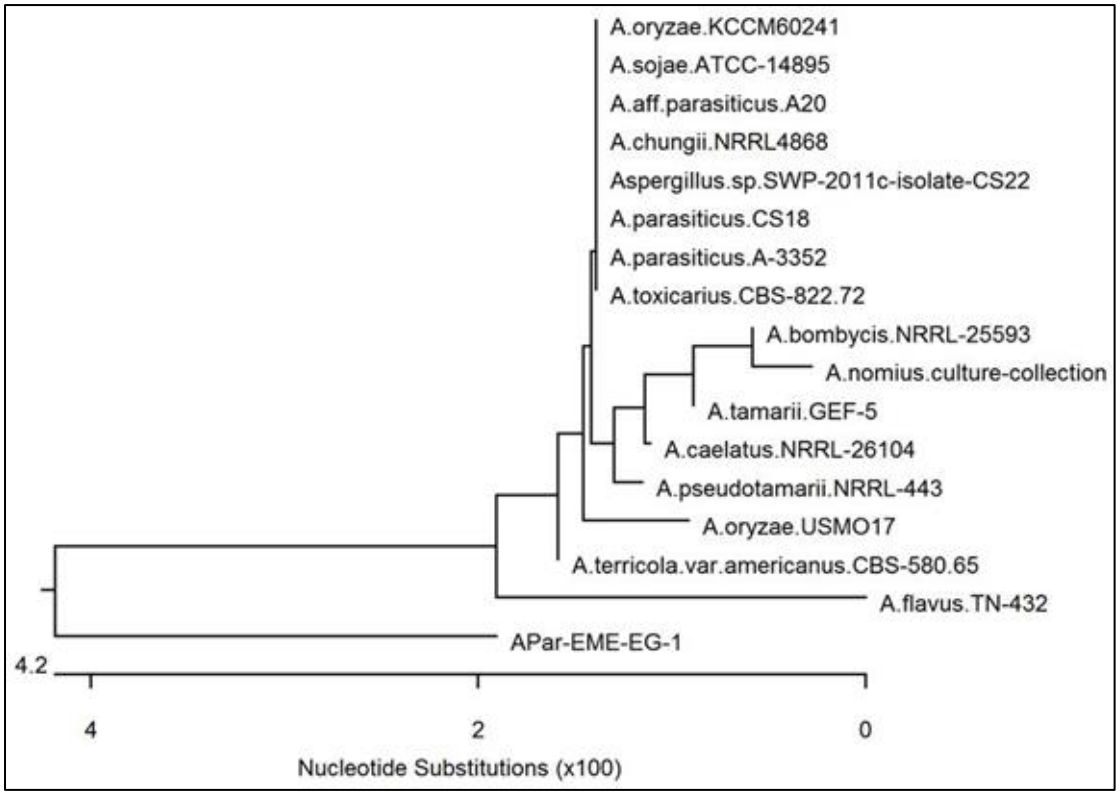

Fig. (2): Phylogenetic tree of A.parasiticus

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1		95.3	95.3	95.3	95.3	95.3	95.5	95.3	95.3	95.0	95.0	94.7	95.7	95.2	94.4	94.9	94.1	1	APar-EME-EG-1
2	4.9		100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.4	99.7	100.0	99.1	99.4	98.8	2	A.parasiticus.A-3352
3	4.9	0.0		100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.4	99.7	100.0	99.1	99.4	98.8	3	A.parasiticus.CS18
4	4.9	0.0	0.0		100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.4	99.7	100.0	99.1	99.4	98.8	4	Aspergillus.sp.SWP-2011c-isolate-CS22
5	4.9	0.0	0.0	0.0		100.0	100.0	100.0	100.0	99.7	99.7	99.4	99.7	100.0	99.1	99.4	98.8	5	A.chungii.NRRL4868
6	4.9	0.0	0.0	0.0	0.0		100.0	100.0	100.0	99.7	99.7	99.4	99.7	100.0	99.1	99.4	98.8	6	A.aff.parasiticus.A20
7	4.6	0.0	0.0	0.0	0.0	0.0		100.0	100.0	99.7	99.7	99.4	99.7	100.0	99.1	99.4	98.8	7	A.terricola.var.americanus.CBS-580.65
8	4.9	0.0	0.0	0.0	0.0	0.0	0.0		100.0	99.7	99.7	99.4	99.7	100.0	99.1	99.4	98.8	8	A.oryzae.KCCM60241
9	4.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0		99.7	99.7	99.4	99.7	100.0	99.1	99.4	98.8	9	A.sojae.ATCC-14895
10	5.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		99.4	99.7	100.0	99.7	99.4	99.1	99.1	10	A.pseudotamarii.NRRL-443
11	5.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.6		99.7	99.4	99.7	99.4	99.1	99.1	11	A.caelatus.NRRL-26104
12	5.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.3	0.3		99.7	99.4	99.7	98.8	99.4	12	A.tamarii.GEF-5
13	4.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	0.6	0.3		99.7	99.4	99.1	99.1	13	A.flavus.TN-432
14	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.6	0.3		99.1	99.4	98.8	14	A.toxicarius.CBS-822.72
15	5.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.6	0.6	0.3	0.6	0.9		98.5	99.7	15	A.bombycis.NRRL-25593
16	5.3	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.9	0.9	1.2	0.9	0.6	1.5		98.2	16	A.oryzae.USMO17
17	6.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.9	0.9	0.6	0.9	1.2	0.3	1.8		17	A.nomius.culture-collection
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		

Divergence

Fig. (3): *Sequences producing significant alignments with Accession in Genbank*

Nucleotide Sequence pair distances of Penicillium ITS2 sequences

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	
1		98.0	98.3	99.0	98.3	92.3	94.0	93.8	92.3	90.8	1
2	2.1		100.0	100.0	100.0	91.8	95.7	95.9	94.4	92.9	2
3	1.7	0.0		100.0	100.0	91.8	95.7	95.8	94.3	92.8	3
4	1.0	0.0	0.0		100.0	92.5	95.6	95.8	94.3	92.8	4
5	1.7	0.0	0.0	0.0		91.8	95.7	95.8	94.3	92.8	5
6	8.1	8.7	8.7	7.9	8.7		87.4	87.5	86.4	84.8	6
7	6.3	4.4	4.4	4.5	4.4	13.8		100.0	94.0	92.3	7
8	6.5	4.2	4.3	4.3	4.3	13.6	0.0		94.2	92.5	8
9	8.1	5.8	5.9	6.0	5.9	15.0	6.3	6.1		92.6	9
10	9.8	7.5	7.6	7.7	7.6	17.0	8.2	7.9	7.8		10
	1	2	3	4	5	6	7	8	9	10	

1 Pen-EME-EG-1
2 Penicillium_purpurogenum.FRR-1061
3 Talaromyces_purpurogenus.IAM13755
4 Penicillium_purpurogenum.CASMB-SEF7
5 Penicillium_sp.ML172
6 Talaromyces_purpurogenus.IAM15392
7 Penicillium_minioluteum.IFV
8 Penicillium_samsonii.CBS-137.84
9 Penicillium_diversum.KUC1284
10 Talaromyces_purpureus.CBS-475.71

Fig. (4): Nucleotide Sequence pair distances of Penicilliumpurpurogenum ITS2 sequences

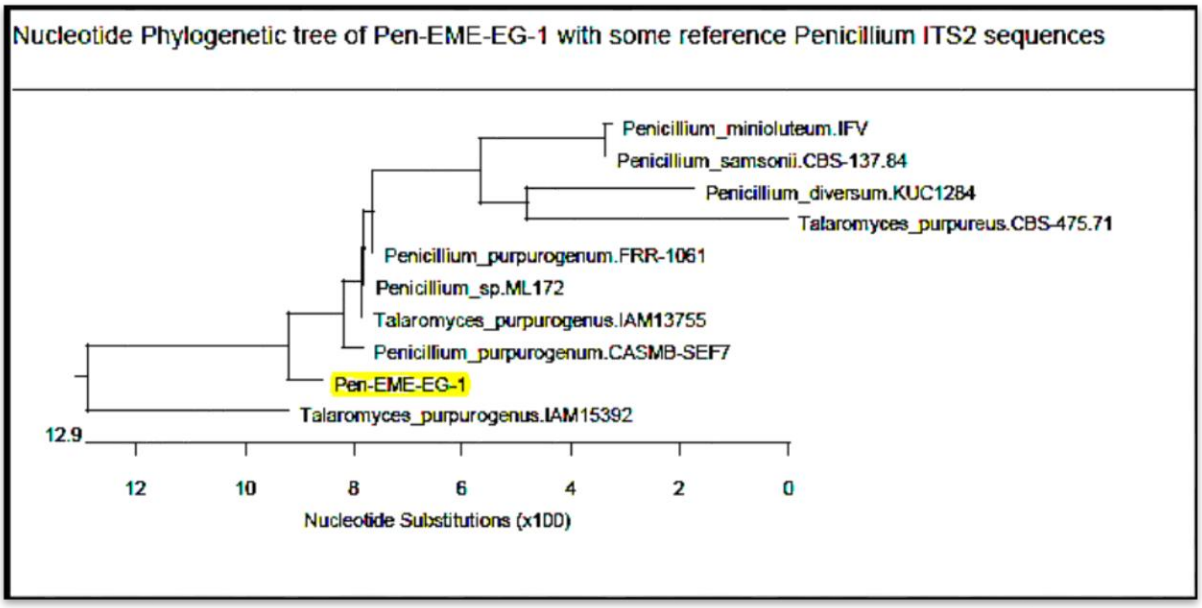

Fig. (5): Nucleotide Phylogenetic tree of Pen-EME-EG-1 with some reference Penicilliumpurpurogenum ITS2 sequences

Fig. (6): *Aspergillusparasiticus* strain A-3352 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: gb|JQ316518.1|Length: 596Number of Matches: 1

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Query  238  -TGACCTCTCATCAGGTAGGGATACCCGTTGAATTTAACTATATC  281
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Sbjct  550  TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATC  594
```

Reverse Aspergillus

Aspergillusparasiticus isolate 1 12B 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

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Query  255  ATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCC  298
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Sbjct  274  ATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCC  231
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Fig. (7):*Penicilliumpurpurogenum* strain FRR 1061 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence ,Sequence ID: gb|AY373926.1|Length: 620Number of Matches: 1Related InformationRange 1: 31 to 603GenBankGraphicsNext

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DownloadGenBankGraphicsNextPreviousDescriptions

Penicilliumpurpurogenum strain CASMB-SEF 7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: gb|JQ663996.1|Length: 585Number of Matches: 1

Related Information

Range 1: 19 to 577GenBankGraphicsNext MatchPrevious Match

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