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ORIGINAL ARTICLE

Molecular Study of Aspirjillus Ochraceus Isolated from Air Condition Unite in Muthanna City

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ABSTRACT

The current study dealt with the isolation and diagnosis of fungi contaminating air condition AC in the laboratories of the Technical Institute in Al-Muthanna, the laboratories of the College of Agriculture at the University of Al-Muthanna during the period from August 14/08/2020 to 28/2/2022 , This study included isolating and diagnosing a fungus from the refrigeration systems and the total number of samples is 400 isolated samples from the refrigeration systems in the city of Muthanna, After the sample was diagnosed phenotypically and microscopically on the medium of Sabouraud Dextrose Agar, it was molecularly diagnosed after extracting DNA for five isolates from the fungus, where all the isolates showed a positive result373bp for molecular.

Keywords: air-conditioning (AC), A.ochraceus, molecular , fungi

INTRODUCTION

Air-conditioning .are a key choice on account of warm conditions, particularly in the somewhat lengthy summer in Iraq, particularly the areas in the southern segments that Iraqi families resort to from February until the finish of October, during which time the growths start to assemble on the channels of air-conditioning whether they are parted air-conditioning .Or standard air-conditioning or vehicle air-conditioning. It is viewed as a damp climate reasonable for the development of fungi[1].

Male [2]fungi are live creatures "The truth of eukaryotic" which is different as far as conduct and establishment as well as nourishment from the other living life forms that are described by sustenance "Heterotrophic" Any that it can make its own food, and the justification behind inability to contain its cells on the "chlorophyll". They have confidence in their food either from the people who are gotten from different living beings or by finishing natural materials Growths has unique parts make them broadly from exercises and occasions that enter human existence.

Parasites spread generally in the indoor and open air climate through air flows as bits of contagious hyphae and spores, and these tiny life forms, including organisms, can enter structures (shut spaces that incorporate homes and government establishments) by entering the outside air into the inside through frameworks Variation and Different Means [3].

Since antiquated times, many investigations applauded the indoor climate organisms, like government organizations and homes, and showed a connection between the presence of contagious spores at an undeniable level in their air and the wellbeing status of individuals in these conditions [4].

Al-Janabi[5] showed that the outside air is the fundamental wellspring of parasites spores, as the shut spots get their inside air from outer sources, through windows, entryways and ventilation openings, so these sources are for the presentation of growths, and the open air and indoor air is seldom liberated from contagious spores since it is the middle person to move and spread starting with one area then onto the next.

Microorganisms caught by the channels can blossom with the channels and can be delivered out of sight, bringing about neurotic structure syndrome[6],Furthermore, it has been resolved that the quantity of organisms present in indoor air is lower than that which colonizes the outer layer of channels utilized in cooling systems52 [7].

Dampness advances contagious development in channel tissues and can likewise help bacterial proliferation which prompts immigration and resulting spread inside indoor environments[8]. This dampness frequently begins from condensate drops that structure cooling towers [9].

Notwithstanding abiotic factors, for example, dust, particulates, divider covers, engineered paints, shines and natural mixtures might add to indoor contamination [10] the contamination comes from sources inside the actual structure, for example, hair shower, aroma, and antiperspirant For room, paints, home devices, air innovation gadgets, printers, and computers[1].

Because of the prior, organisms have become risky on the grounds that they hurtfully affect human wellbeing, and openness to parasites causes sensitivities, aggravations and other harmful impacts [12].The microbial development in air-conditioning frameworks prompts contamination in indoor air quality, which causes It has numerous medical conditions [13].

Mashat[14] referenced that the method involved with breathing in pieces of the contagious hyphae or the spores of the parasites causes sensitivity contamination and different sicknesses, including harming. This relies upon the sort of organism and the time-frame of openness to it. Additionally, unfavorably susceptible reactions (nasal sensitivities, extreme touchiness pneumonia, and asthma) can be counted. It is one of the regular and normal issues for an enormous scope, and it is connected with the inward breath of growths in the air.

MATERIALS AND METHODS

Samples: 400samples were collected from air contaminated by sterial swabs and cultured directly on fungal specific medium

Primers: PCR primers were designed in this study using NCBI-Genbank database and primer3 plus online. These primers were provided by Macrogen company from Korea as following table (1)

PCR detection gene primers with their nucleotide sequence and product size.

Table 1: PCR detection gene primers with their nucleotide sequence and product size.

Primer	Sequence		PCR product size	Genbank code
Aspergillus ochraceus 18S rRNA gene	F	CTTCCTTAGGGGTG GCACAG	373bp	LN809077.1
	R	CCTACAAGAGCGG GTGACAA		

Fungal DNA extraction: Fungal genomic DNA was extracted from A. niger isolates by using (G-Spin DNA extraction kit with modification**.**

PCR product analysis

The PCR products were analyzed by agarose gel electrophoresis method as following steps:

1 1.5% Agarose gel was prepared in using 0.5X TBE and dissolving in microwave for 5 minutes, and left to cool for 50°C.

2 Then 3µl ethidium bromide stain were added into agarose gel solution.

3 Agarose gel solution was poured in tray after fixed the comb in proper position and left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray.

4 The gel tray was fixed in electrophoresis chamber and filled by 0.5X TBE buffer.

5 10µl PCR product were loaded in to each well with added 3µl (DNA marker Ladder) in first well. Then electric current was performed at 100 volt and 80 AM for 1hour.

5- PCR products were visualized by using UV Transilluminator.

RESULT AND DISCUSSION

The review affirmed the most presence of the sort Aspergillus, and it was addressed by the species A.niger, A.flavus, A. candidus, A.clavtus, A.terreus, A.Aochracues, A.versicolor, A.fumigatus, at (11,27,10,28, 29,184,52,44) separates individually. The outcomes likewise incorporated the confinement and conclusion of a few animal groups having a place with the sort Penicillum, and the quantity of disconnects added up to (79) segregates. From a similar table beneath, it was noticed that species having a place with the variety Cladosporium showed up with various confines (20) as the outcomes showed the development of some of different growths are Fusarium .spp, Trachophyton rubrum, Mucor racemosus Cladosporium sphaerospermum, Alternaria alternat, Candida albcans, , Rhizopus spp, and the quantity of disconnects added up to 38,20,24,58,63,32,46) separately, and these outcomes are predictable with these outcomes With what was reached by[20] and it disagrees with what was reached by[21].where Penicillum scored. The most elevated rate was 40%, and Aspergillus scored 38.66%,

The Molecular study of fungi Aspergillus ochraceus by using species primer showed significantly results as showed in Figure 1 and our study similar to that provided by

Molecular identification: The diagnosis was confirmed by genotyping, whereby polymerase chain reaction (PCR) technique was used to confirm the diagnosis by microscopic examination of A.ochraceus. The results of electrophoresis on Agarose gel of DNA samples extracted from the fungus under study showed that the 18S rRna gene initiator was about 373bp.

It is noted in Figure(1) the presence of the 373 bp band, which confirms that the suspected fungus belongs to A.ochraceus, and this confirms the validity of the traditional preliminary diagnosis of the fungus under study. These results are in agreement with Helu (2021) in the identification of 11 different isolates of cereal grains. Different isolated from grains.

It is considered one of the modern techniques that give accurate information in the field of diagnosis, and it depends on doubling the size of the genetic material and matching it with the DNA of original isolates whose genetic makeup has not been changed. Isolated from different disease states (Lateage, 2001).

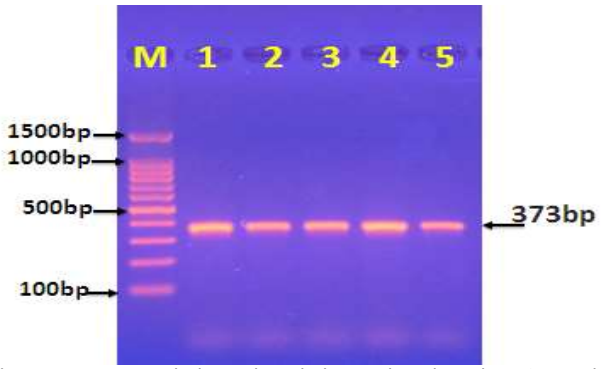

Figure 1: Agarose gel electrophoresis image that show the PCR product analysis of 18S rRNA gene in Aspergillus ochraceus positive isolate, where ladder (1500-100bp), and lane (1-5) were showed positive PCR at (373bp) PCR product size.

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