https://doi.org/10.46389/rjd-2024-649
www.jrdiabet.ro
Rom J Diabetes Nutr Metab Dis
2024; Volume 31, Issue 1, pages 649-658

TYPES OF ASPERGILLUS FUNGI ISOLATED FROM THE AIR OF THE AREA BETWEEN THE TWO HOLY MOSQUES IN HOLY KARBALA

Mohamed Hakam Atwan, Prof. Ban Taha Mohammad
College of Education for Pure Sciences, University of Karbala, Iraq
e03161372@s.uokerbala.edu.iq ban.taha@uokerbala.edu.iq

Abstract
The study included molecular diagnosis of some types of Aspergillus fungi in specific areas between the two holy mosques of the city of Karbala. With the aim of identifying the types of Aspergillus present in the region and their importance to community health. Samples were taken from the atmospheric air using an air-sampler system. Using the technique of random polymorphic DNA replication (genetic fingerprinting) after the DNA was extracted from each isolate at a final concentration of pb (524) per 2-3 grams of wet mycelium with 100% purity, using primers ITS1 and ITS4, and by electrophoresis through a gel. Agarose with a concentration of 1.5%, dyed with ethidium bromide, and photographed under ultraviolet light. The genetic fingerprint of four isolates isolated from the air was determined by the appearance of DNA duplication bands for each isolate with one or more primers, and they included: 1. Aspergillus fumigatus, A.fumigatus 2, A. terreus were registered in the Global GenBank with serial numbers (OR492482. OR492612. OR497754) respectively, with a similarity rate of 100% with global fungi.

Keyword: Fungi Molecular diagnosis of fungi, Aspergillus spp.

Introduction
Fungi are eukaryotic organisms, the number of species recorded so far has reached about 100,000 fungal species, at a rate of 1,200,000 fungal species annually (Kirk et al., 2008). Many genera of fungi constitute one of the most important problems that exist at the present time due to their wide spread in nature, and they are considered one of the most important main causes that cause bacterial spoilage of foodstuffs (Gizachew et al., 2016). Fungi also cause diseases in humans, leading to more deaths. Of the 1.5-2 million annually, more than one billion people suffer from acute fungal diseases (Kirk et al., 2008), and previous studies indicate that fungi are among the microorganisms that most threaten animals and plants, and constitute about 65% of infections compared to other factors. Other pathogens (Fisher et al., 2012) and in light of these circumstances, the phenomenon of global warming and the climate changes accompanying it have led to an increase in the incidence of many fungal diseases (Ostrosky-Zeichner et al., 2010). Therefore, concerns have emerged about the occurrence of epidemics that may be caused by them. Fungal pathogens in the twenty-first century are linked to climate change and weakened immunity, which leads to increased resistance and difficulty resolving health problems resulting from some of the current associations between chronic diseases and fungal infections, which greatly enhances the medical importance of fungi and the necessity of human preparedness to confront fungal infections, which requires further research. In the field of finding alternatives to manufactured antibiotics (Albuquerque & Casadevall, 2012), infections caused by spores or fungal filaments spread in the air constitute a global health problem for humans around the world, as inhaling airborne dust

containing fungal spores causes many diseases, including Bronchopulmonary aspergillosis, aspergillitis in the sinuses, and invasive aspergillosis, which is the most dangerous and uncommon infection that affects the lungs or other body systems and is the main cause of death in individuals who suffer from immunodeficiency (Badran et al., 2018). The fungal species present in Air plays an important role in respiratory allergies and asthma (Mezzari et al., 2002). Fungi are not limited to allergies, asthma, and respiratory system diseases, but go beyond that, such as cancers, as well as what is known as Aspergillosis, which is caused by some species of fungi (Aspergillus). It is considered a group of genus fungi. Aspergillus is one of the most widespread species in most environments and is one of the most important producers of aflatoxins (Balina et al., 2018). Therefore, concerns have emerged about the occurrence of epidemics that may be caused by fungal pathogens in the twenty-first century, linked to climate change and weak immunity, which leads to increased resistance and difficulty in resolving Health problems resulting from some current associations between chronic diseases and fungal infections, which greatly enhances the medical importance of fungi and the necessity of human preparedness to confront fungal infections, which requires further research in the field of finding alternatives to manufactured antibiotics. Many studies conducted inside Iraq have confirmed the presence of fungal pollution in the air, including a study of fungal pollution of the air in the city of Hilla, which proved the spread of fungi in the air and the dominance of Aspergillus fumegato species ((Khairallah & Jawad, 2017). A comparative study of air fungi in the city of Karbala was conducted, which proved the presence and spread of Fungi. The study confirmed in its comparison the spread of the genus Aspergillus in the air and its dominance in that region (Muhammad & Muhammad, 2007). The group of fungi of the genus Aspergillus is considered, taking into account the studies mentioned above and the high temperatures that the city of Karbala enjoys due to its sandy soil and the spread of fungi in dust particles. Airborne, and given the lack of research on airborne fungi in Iraq in general and in Karbala in particular, the research focuses on airborne fungi in specific areas of the city and the extent of their impact on the population. The research was conducted during the month of October 2022, to try to reduce or limit the spread of pollution from During health awareness, guidance and advice, and the use of means to sterilize and disinfect the atmosphere, it was found that the use of traditional methods is insufficient in most cases, due to the asymmetrical phenotype and multiplicity of shapes, as well as the difference in environmental conditions.

Materials and working methods:-

The research was conducted during the period of October 2022 in specific areas between the Two Holy Mosques in the city of Karbala.

1- Prepare the medium (potato-dextrose-acchar (PDA), according to the manufacturer's instructions (HIMEDIA-India), by dissolving 39 g of the crushed medium in one liter of distilled water to which the antibiotic chloramphenicol has been added at a concentration of 502 mg/l. The medium was sterilized. In an autoclave at a temperature of 121 degrees Celsius and under a pressure of 1.5 atmospheres for 15 minutes, after cooling, pour the medium into plastic Petri dishes.

2- Samples were collected using a steel air-sampler system manufactured by Merck KGaA (KGaA), by placing the dish containing the Potato-Dextrose-Agar (PDA) medium. The lid was removed from the dish, placed inside the nozzle of the device, closed tightly, and turned on. The device is carried out in accordance with the manufacturer's terms and conditions, and the device must be walked at a height of 1.5 meters above ground level for 60 seconds, after which the process of withdrawing microbes from the air is stopped by pressing the stop button and removing the dish from inside the device, and the dishes are covered with their lids that were kept in sterile paper.
3- The dishes were taken to the laboratory for incubation at a temperature of 38°C, and after a week of incubation, the growing fungi were diagnosed according to what was stated in (Ellis et al., 2014). The isolates were purified several times in order to obtain pure fungal isolates.
4- The isolated fungi were diagnosed traditionally and then using PCR technology and using:

First- DNA extraction method: The test was carried out according to the manufacturer's instructions (FAVORGEN) to extract the fungal DNA, as follows: - 100 microliters of the fungal culture was taken and 1 ml of FA Buffer was added to the fiber fraction to obtain the DNA. TG1 Buffer and TG2 Buffer were added to the samples and mixed, and after some additions and dilutions, DNA was obtained that can be stored or preserved between 4 and -20 °C. Promega's KIT PCR method (Green Master MIX) was performed according to the manufacturer's protocol. After DNA isolation, quantification was performed using Macrogen (Korean ITS 4 and ITS1) specific primers were used and PCR analysis was processed. Specific primers (nucleotide primers, Prime) were used to identify TCCGTAGGTGAACCTGCGG Forward primer and Reverse primer: TCCTCCGCTTATTGATATGC.

Second: Analysis of the results of the PCR test. The PCR test was performed according to the (KIT) method of Promega, where Green Master MIX and two types of special primers (ITS 4 and Microgen (ITS1) were used for all fungi as shown in (Table 1). Electrophoresis was performed. For agarose gel. Using 1.5% agarose gel to read the polymerase reaction result, sequence analysis of the PCR product is as follows: (Mishra et al., 2010) 1.5-2 µg of agarose gel was dissolved in 100 ml of TBE buffer solution at a concentration of 1 Then heat it for 5 minutes using the microwave, then leave the gel to cool at a temperature of 50 °C. Then 3 µl of ethidium bromide DNA dye was added and mixed well with the gel. After that, the agarose gel was poured into a rectangular basin containing a comb to determine the PCR samples. Then the gel was allowed to solidify. At room temperature for 15 minutes, the comb was then carefully removed from the gel and transferred to the electrophoresis bath. Samples for the PCR product were loaded and placed in the holes of the gel. A DNA 100 ladder was used to measure the PCR product and placed in the first hole. Samples were then placed DNA in the remaining holes.The agarose gel was immersed in buffer solution in the center and then the migration was run at 80 V, 58 A, for 75 min. After migration, the gel containing the PCR product was scanned using UV gel documentation to determine the product per unit of measurement (Sambrook & Russell, 2006).

Table 1 The specific primers ITS 1 and ITS4 MICROGEN KOREA were used

Primers	Sequence		Amplicon
ITS 1	F	TCCGTAGGTGAACCTGCGG	524bp
ITS 4	R	TCCTCCGCTTATTGATATGC	

5- The PCR results were then sent to the Macrogen company in South Korea for the purpose of determining the sequence of the nitrogenous bases for each fungal sample. The data received from the company was evaluated and analyzed using the Chromas program, and for the purpose of determining the similarity between the studied mushrooms and the fungi recorded internationally, the Basic Local Alignment Search Tool program was used. (BLAST) of the National Center for Biotechnology Information website. Search results: National Center for Biotechnology Information.

Results and discussion

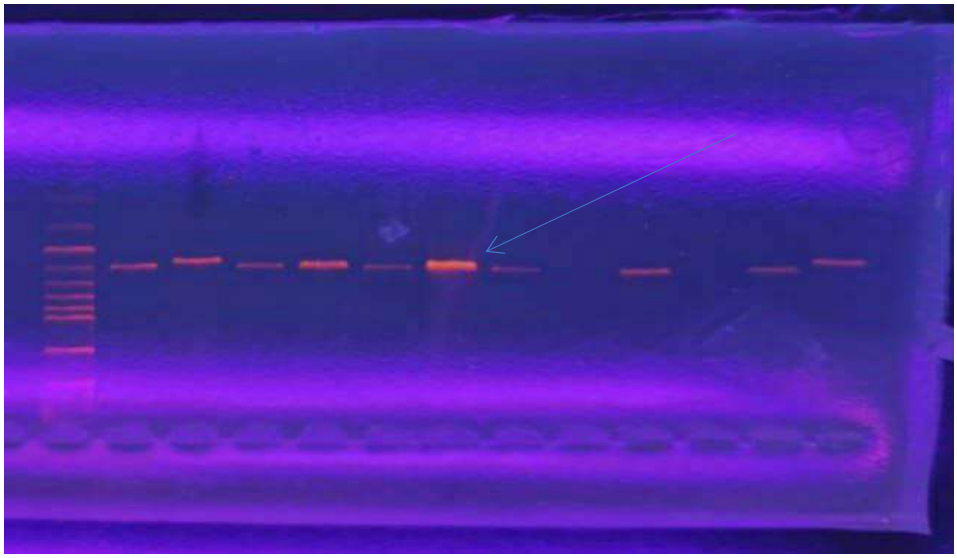


Figure (1) shows the process of electrophoresis (EIectrophoresis) on a 1.5% agarose gel at a voltage of 100 for an hour for isolates of the genus Aspergillus with a PCR product of size pb524.

Detection using (P.C.R) technology

A - Analysis of the sequences of the amplification products. The amplified gene products were sent to the Korean company Macrogen to determine the sequences of the nitrogenous bases. These sequences were approved by comparing them with the information available about this gene on the website of the National Center for Biotechnology Information (NCBI) through the website (https://www.ncbi.nlm.nih.gov/nuccore) According to the BLAST Nucleotide program, in order

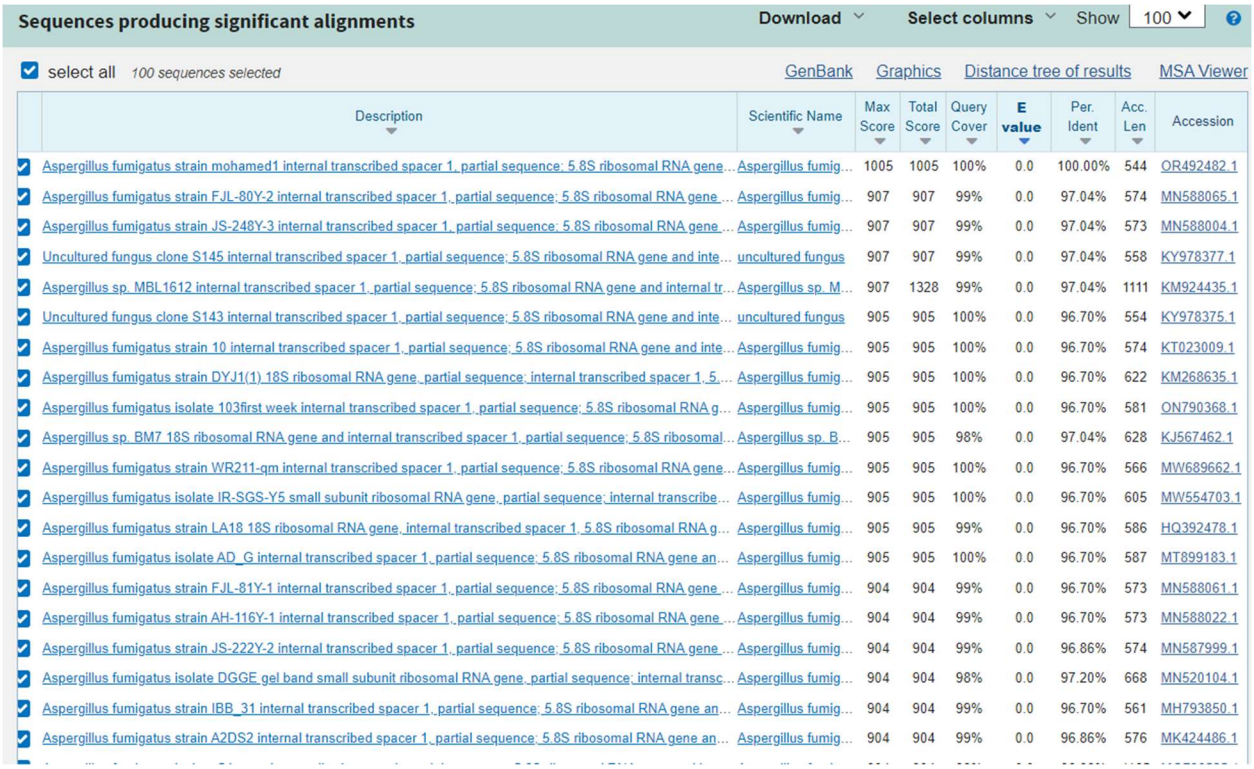
to identify the type of the selected isolate, the base sequences were recorded at NCBI by filling out the form for registering the ITS18 gene to obtain a special accession number for the local isolate. The Phylogenetic tree was also drawn for the local isolate after matching it to closely related strains in NCBI and based on the Blast Tree View program. The results of the isolates showed a 100% match with the international isolates, as in Table No. (3). It was considered the first registration of the isolates in the GenBank. Special numbers were given as shown in Table No. (2).

Table No. (2) shows the numbers registered in the GenBank for genetically diagnosed fungal isolates.

fungi type	Isolate number in GenBank
Aspergillus fumigatus	OR492482
Aspergillus fumigatus	OR492612
Aspergillus terreus	OR497754

The interspacer region ITS1 and ITS4 in the ribosomal gene S18 is considered stable and is used successfully in distinguishing different types of fungi and gives decisive results in diagnosis (Holland, 2010).

B- Determining the sequence of the nitrogenous bases and analyzing the bioinformatics and genetic tree Phylogeny showed the results of the analysis of the nitrogenous base sequence (Nucleotide sequence) of the duplicated DNA bands using the NCBI program and comparing it with the data available at the National Center for Biotechnology Information (NCBI). The four isolates sent, each of them belonging to the isolate bearing the number OR 492482 and registered in the GenBank, belong to the fungus Aspergillus fumigatus, where the match rate with international isolates was 100%, as shown in Figure No. (1-1).



Sequences producing significant alignments Download Select columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aspergillus fumigatus strain mohamed1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aspergillus fumig...	1005	1005	100%	0.0	100.00%	544	OR492482.1
Aspergillus fumigatus strain FJL-80Y-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Aspergillus fumig...	907	907	99%	0.0	97.04%	574	MN588065.1
Aspergillus fumigatus strain JS-248Y-3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Aspergillus fumig...	907	907	99%	0.0	97.04%	573	MN588004.1
Uncultured fungus clone S145 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...	uncultured fungus	907	907	99%	0.0	97.04%	558	KY978377.1
Aspergillus sp. MBL1612 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tr...	Aspergillus sp. M...	907	1328	99%	0.0	97.04%	1111	KM924435.1
Uncultured fungus clone S143 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...	uncultured fungus	905	905	100%	0.0	96.70%	554	KY978375.1
Aspergillus fumigatus strain 10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...	Aspergillus fumig...	905	905	100%	0.0	96.70%	574	KT023009.1
Aspergillus fumigatus strain DYJ1(1) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5....	Aspergillus fumig...	905	905	100%	0.0	96.70%	622	KM268635.1
Aspergillus fumigatus isolate 103first week internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA g...	Aspergillus fumig...	905	905	100%	0.0	96.70%	581	ON790368.1
Aspergillus sp. BM7 18S ribosomal RNA gene and internal transcribed spacer 1, partial sequence; 5.8S ribosomal...	Aspergillus sp. B...	905	905	98%	0.0	97.04%	628	KJ567462.1
Aspergillus fumigatus strain WR211-qm internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aspergillus fumig...	905	905	100%	0.0	96.70%	566	MW689662.1
Aspergillus fumigatus isolate IR-SGS-Y5 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Aspergillus fumig...	905	905	100%	0.0	96.70%	605	MW554703.1
Aspergillus fumigatus strain LA18 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA g...	Aspergillus fumig...	905	905	99%	0.0	96.70%	586	HQ392478.1
Aspergillus fumigatus isolate AD_G internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Aspergillus fumig...	905	905	100%	0.0	96.70%	587	MT899183.1
Aspergillus fumigatus strain FJL-81Y-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Aspergillus fumig...	904	904	99%	0.0	96.70%	573	MN588061.1
Aspergillus fumigatus strain AH-116Y-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aspergillus fumig...	904	904	99%	0.0	96.70%	573	MN588022.1
Aspergillus fumigatus strain JS-222Y-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Aspergillus fumig...	904	904	99%	0.0	96.86%	574	MN587999.1
Aspergillus fumigatus isolate DGGE gel band small subunit ribosomal RNA gene, partial sequence; internal transc...	Aspergillus fumig...	904	904	98%	0.0	97.20%	668	MN520104.1
Aspergillus fumigatus strain IBB_31 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Aspergillus fumig...	904	904	99%	0.0	96.70%	561	MH793850.1
Aspergillus fumigatus strain A2DS2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Aspergillus fumig...	904	904	99%	0.0	96.86%	576	MK424486.1

Figure (1-1) shows the conformity of the Aspergillus fumigatus isolate with global isolates in GenBank.

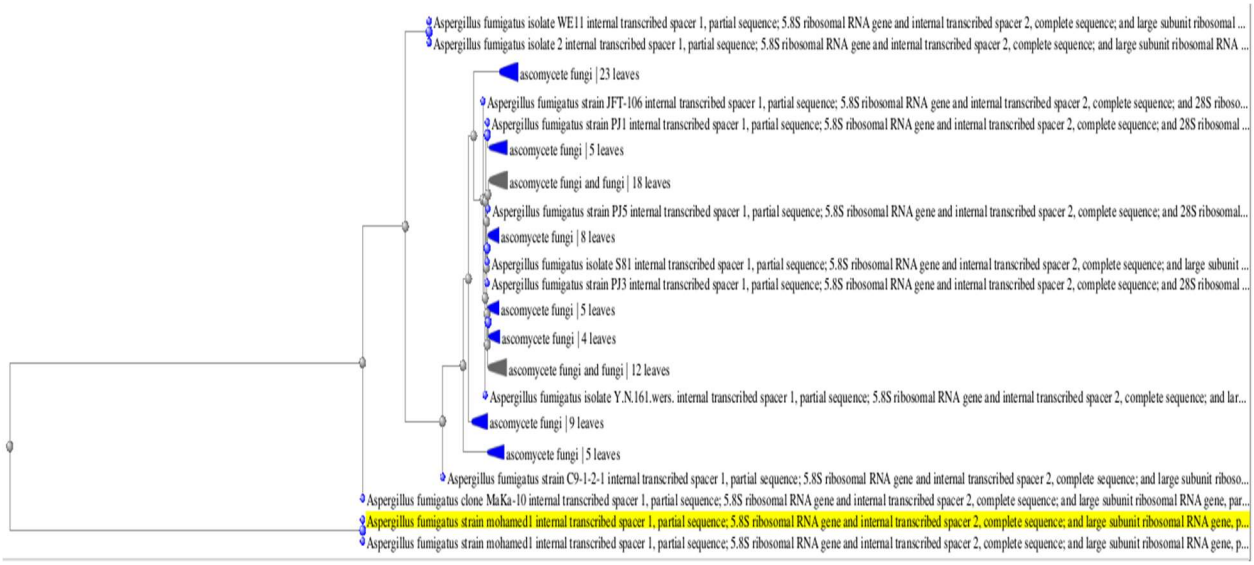


Figure (2-1): The genetic tree of the fungus Aspergillus fumigatus (marked in yellow), which was created based on the sequences of its nitrogenous bases for the ITS-rDNA region, in addition to the sequences of known international strains of the same fungus obtained from the GenBank data repository. Genetic distances were calculated using the neighbor-joining method.

The isolate bearing the number OR 492612 and registered in the GenBank belongs to the fungus

Aspergillus fumigatus, and the match rate with global isolates was 100%, as shown in Figure No. (1-3).

Sequences producing significant alignments Download Select columns Show 100

select all 100 GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aspergillus fumigatus strain Mohamed.Hakam.Atwan internal transcribed spacer 1, partial sequence; 5.8S riboso...	Aspergillus fumig...	1024	1024	100%	0.0	100.00%	554	OR492612.1
Aspergillus fumigatus strain Hussain8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, ...	Aspergillus fumig...	658	658	97%	0.0	88.79%	557	OR243757.1
Aspergillus fumigatus strain Hussain5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, ...	Aspergillus fumig...	652	652	97%	0.0	88.64%	561	OR243749.1
Aspergillus fumigatus strain Hussain10 small subunit ribosomal RNA gene, partial sequence; internal transcribed ...	Aspergillus fumig...	645	645	94%	3e-180	89.04%	567	OR243759.1
Aspergillus fumigatus isolate 224_1_4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Aspergillus fumig...	640	640	95%	1e-178	88.60%	601	MW789036.1
Aspergillus fumigatus isolate 1110009L1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Aspergillus fumig...	638	638	95%	5e-178	88.58%	546	MN559667.1
Aspergillus fumigatus isolate DGGE gel band small subunit ribosomal RNA gene, partial sequence; internal transc...	Aspergillus fumig...	638	638	95%	5e-178	88.56%	577	MN519789.1
Aspergillus fumigatus isolate F11 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Aspergillus fumig...	638	638	95%	5e-178	88.56%	653	MK816855.1
Aspergillus fumigatus strain SSH01 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and...	Aspergillus fumig...	638	638	95%	5e-178	88.58%	608	KT266801.1
Aspergillus fumigatus isolate ZW-L-20 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Aspergillus fumig...	638	638	95%	5e-178	88.58%	602	OP482428.1
Aspergillus fumigatus strain IITRSC519 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aspergillus fumig...	638	638	94%	5e-178	88.83%	584	MT989355.1
Aspergillus fumigatus strain Hussain4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Aspergillus fumig...	636	636	96%	2e-177	88.19%	571	OR243745.1
Aspergillus sp. MBL1412 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tr...	Aspergillus sp. M...	636	636	95%	2e-177	88.56%	1110	KM924434.1
Aspergillus fumigatus strain AHBR7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Aspergillus fumig...	636	636	95%	2e-177	88.43%	942	KF305746.1
Aspergillus fumigatus isolate MEBP0074 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Aspergillus fumig...	634	634	95%	7e-177	88.41%	728	MT597427.1
Aspergillus fumigatus isolate MEBP0062 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Aspergillus fumig...	634	634	94%	7e-177	88.64%	651	MT593013.1
Aspergillus fumigatus isolate 1110080L1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Aspergillus fumig...	634	634	95%	7e-177	88.41%	596	MN559668.1
Aspergillus fumigatus clone M3Ai 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ...	Aspergillus fumig...	634	634	94%	7e-177	88.64%	707	MH378448.1
Aspergillus fumigatus strain CMXY2576 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aspergillus fumig...	634	634	94%	7e-177	88.64%	581	MG991582.1
Aspergillus fumigatus isolate F2-3-43 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...	Aspergillus fumig...	634	634	95%	7e-177	88.41%	599	KX349486.1

Figure (1-3) shows the conformity of the Aspergillus fumigatus isolate with global isolates in GenBank.

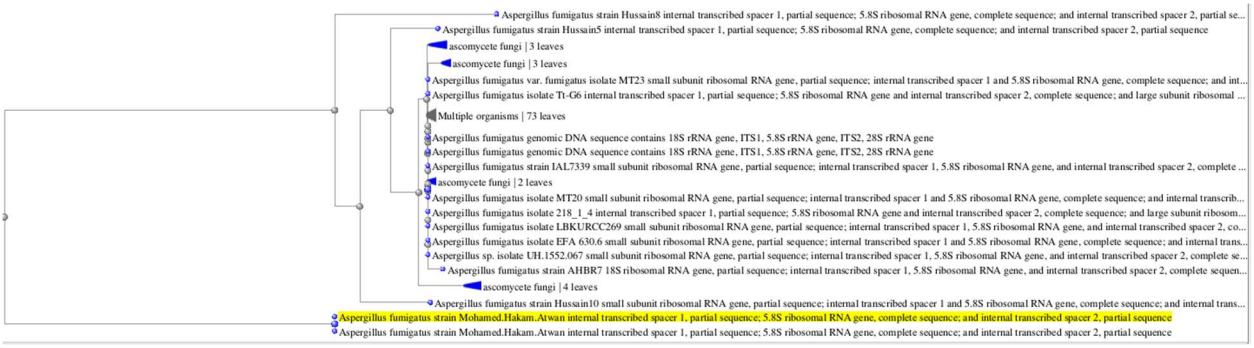
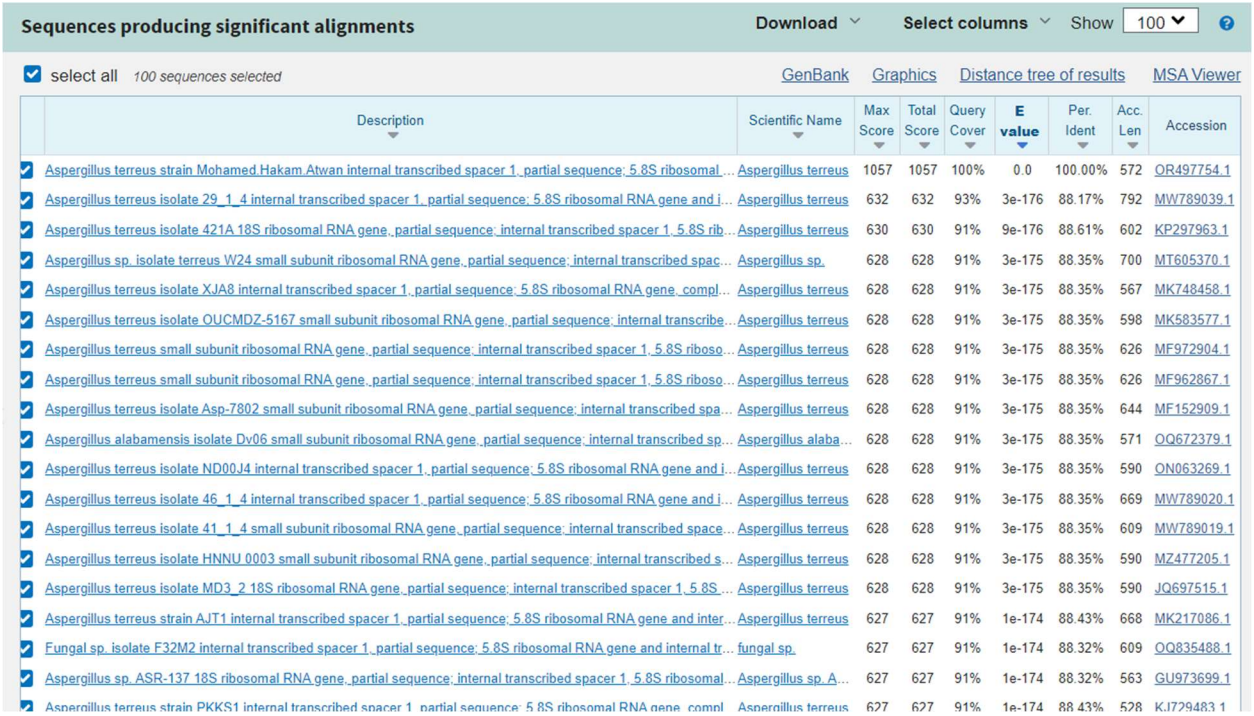


Figure (1-4): Genetic tree of the fungus Aspergillus fumigatus (marked in yellow), which was created based on the sequences of its nitrogenous bases for the ITS-rDNA region, in addition to the sequences of known international strains of the same fungus obtained from the GenBank data repository. Genetic distances were calculated using the neighbor-joining method.

The isolate bearing the number OR 497754 and registered in the GenBank belongs to the fungus Aspergillus terreus, where the match rate with global isolates was 100%, as shown in Figure No. (1-5).



Sequences producing significant alignments Download Select columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aspergillus terreus strain Mohamed.Hakam.Atwan internal transcribed spacer 1, partial sequence; 5.8S ribosomal ...	Aspergillus terreus	1057	1057	100%	0.0	100.00%	572	OR497754.1
Aspergillus terreus isolate 29_1_4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Aspergillus terreus	632	632	93%	3e-176	88.17%	792	MW789039.1
Aspergillus terreus isolate 421A 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rib...	Aspergillus terreus	630	630	91%	9e-176	88.61%	602	KP297963.1
Aspergillus sp. isolate terreus W24 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Aspergillus sp.	628	628	91%	3e-175	88.35%	700	MT605370.1
Aspergillus terreus isolate XJA8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, compl...	Aspergillus terreus	628	628	91%	3e-175	88.35%	567	MK748458.1
Aspergillus terreus isolate OUCMDZ-5167 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Aspergillus terreus	628	628	91%	3e-175	88.35%	598	MK583577.1
Aspergillus terreus small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S riboso...	Aspergillus terreus	628	628	91%	3e-175	88.35%	626	MF972904.1
Aspergillus terreus small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S riboso...	Aspergillus terreus	628	628	91%	3e-175	88.35%	626	MF962867.1
Aspergillus terreus isolate Asp-7802 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Aspergillus terreus	628	628	91%	3e-175	88.35%	644	MF152909.1
Aspergillus alabamensis isolate Dv06 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Aspergillus alaba...	628	628	91%	3e-175	88.35%	571	OQ672379.1
Aspergillus terreus isolate ND00J4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Aspergillus terreus	628	628	91%	3e-175	88.35%	590	ON063269.1
Aspergillus terreus isolate 46_1_4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Aspergillus terreus	628	628	91%	3e-175	88.35%	669	MW789020.1
Aspergillus terreus isolate 41_1_4 small subunit ribosomal RNA gene, partial sequence; internal transcribed space...	Aspergillus terreus	628	628	91%	3e-175	88.35%	609	MW789019.1
Aspergillus terreus isolate HNNU 0003 small subunit ribosomal RNA gene, partial sequence; internal transcribed s...	Aspergillus terreus	628	628	91%	3e-175	88.35%	590	MZ477205.1
Aspergillus terreus isolate MD3_2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ...	Aspergillus terreus	628	628	91%	3e-175	88.35%	590	JQ697515.1
Aspergillus terreus strain AJT1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inter...	Aspergillus terreus	627	627	91%	1e-174	88.43%	668	MK217086.1
Fungal sp. isolate F32M2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tr...	fungal sp.	627	627	91%	1e-174	88.32%	609	OQ835488.1
Aspergillus sp. ASR-137 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal ...	Aspergillus sp. A...	627	627	91%	1e-174	88.32%	563	GU973699.1
Aspergillus terreus strain PKKS1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, compl...	Aspergillus terreus	627	627	91%	1e-174	88.43%	528	KJ729483.1

Figure (1-5) shows the conformity of the Aspergillus terreus isolate with global isolates in GenBank.

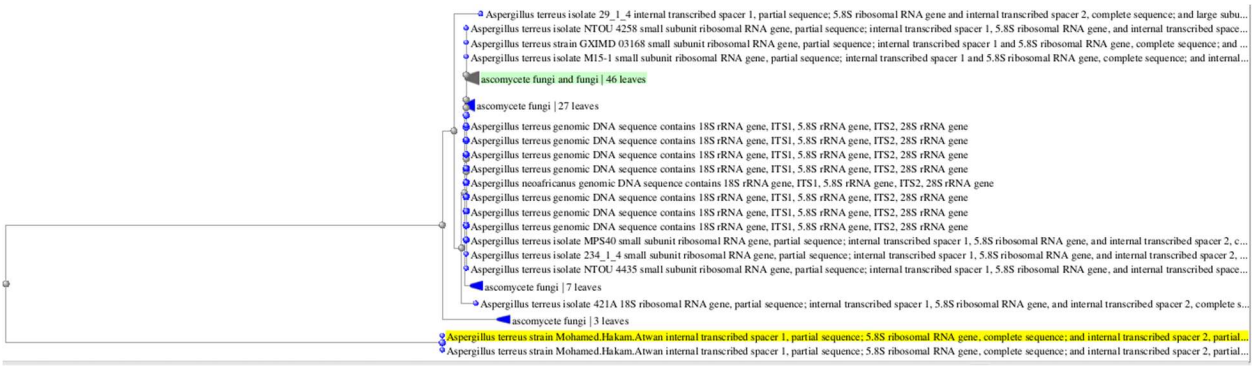


Figure (1-6): Genetic tree of the fungus Aspergillus terreus (marked in yellow), which was constructed based on the sequences of its nitrogenous bases for the ITS-rDNA region, in addition to the sequences of known international strains of the same fungus obtained from the GenBank data repository. Genetic distances were calculated using the neighbor-joining method.

Table (3) shows the percentage of genetic identity between the local isolates under study and the global isolates registered in the fungal gene bank.

Species Local aerobic fungi	Number Join the gene bank	Sequences of homologous species in NCB_BLAST		
		Types of global aerobic fungi	GenBank accession number	Percentage match

Aspergllus fumigatus	OR492482	Aspergllus fumigatus	MN588065	%100
Aspergllus fumigatus	OR492612	Aspergllus fumigatus	OR243757	%100
Aspergllus terreus	OR497745	Aspergllus terreus	MW789039	%100

The results of the analysis showed a clear convergence in the local fungal species with the rest of the other global species shown in an analysis of the genetic tree.

References

Albuquerque, P., & Casadevall, A. (2012). Quorum sensing in fungi–a review. *Medical Mycology*, *50*(4), 337–345.

Balina, A., Kebede, A., & Tamiru, Y. (2018). Review on aflatoxin and its impacts on livestock. *Journal of Dairy and Veterinary Sciences*, *6*(2), e555685.

Ellis, M. L., Jimenez, D. R. C., Leandro, L. F., & Munkvold, G. P. (2014). Genotypic and phenotypic characterization of fungi in the Fusarium oxysporum species complex from soybean roots. *Phytopathology*, *104*(12), 1329–1339.

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, *484*(7393), 186–194.

Gizachew, D., Szonyi, B., Tegegne, A., Hanson, J., & Grace, D. (2016). Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. *Food Control*, *59*, 773–779.

Holland, S. M. (2010). Chronic granulomatous disease. *Clinical Reviews in Allergy & Immunology*, *38*, 3–10.

Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). Ainsworth & Bisby's Dictionary of the fungi. CAB International. *Wallingford, United Kingdom*.

Mezzari, A., Perin, C., Santos Junior, S. A., & Bernd, L. A. G. (2002). Airborne fungi in the city of Porto Alegre, Rio Grande do Sul, Brazil. *Revista Do Instituto de Medicina Tropical de São Paulo*, *44*, 269–272.

Mishra, V., Nag, V. L., Tandon, R., & Awasthi, S. (2010). Response surface methodology-based optimisation of agarose gel electrophoresis for screening and electropherotyping of rotavirus. *Applied Biochemistry and Biotechnology*, *160*, 2322–2331.

Ostrosky-Zeichner, L., Casadevall, A., Galgiani, J. N., Odds, F. C., & Rex, J. H. (2010). An insight into the antifungal pipeline: selected new molecules and beyond. *Nature Reviews Drug Discovery*, *9*(9), 719–727.

Sambrook, J., & Russell, D. W. (2006). Cycle Sequencing: Dideoxy-mediated Sequencing Reactions Using PCR and End-labeled Primers. *Molecular Cloning: A Laboratory Manual,*

2, 12–51.

Badran, B., Abd, H. A., & Ramadan, N. a. (2018). Isolating and diagnosing air fungi inside and outside Tikrit city school buildings. Dirasat Mosiliya, 203–211.

Khairallah, A. A., & Jawad, Z. K. (2017). Study of fungal pollutants in the air of Hilla city. Al-Kufa University Journal for Biology, 9(1).

Muhammad, b. T., & Muhammad, L. i. (2007). Comparative Study of Fungi in the Air of Kerbala City. Journal of Kerbala University, 5(4).

Albuquerque, P., & Casadevall, A. (2012). Quorum sensing in fungi–a review. *Medical Mycology*, *50*(4), 337–345.

Balina, A., Kebede, A., & Tamiru, Y. (2018). Review on aflatoxin and its impacts on livestock. *Journal of Dairy and Veterinary Sciences*, *6*(2), e555685.

Ellis, M. L., Jimenez, D. R. C., Leandro, L. F., & Munkvold, G. P. (2014). Genotypic and phenotypic characterization of fungi in the Fusarium oxysporum species complex from soybean roots. *Phytopathology*, *104*(12), 1329–1339.

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, *484*(7393), 186–194.

Gizachew, D., Szonyi, B., Tegegne, A., Hanson, J., & Grace, D. (2016). Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. *Food Control*, *59*, 773–779.

Holland, S. M. (2010). Chronic granulomatous disease. *Clinical Reviews in Allergy & Immunology*, *38*, 3–10.

Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). Ainsworth & Bisby's Dictionary of the fungi. CAB International. *Wallingford, United Kingdom*.

Mezzari, A., Perin, C., Santos Junior, S. A., & Bernd, L. A. G. (2002). Airborne fungi in the city of Porto Alegre, Rio Grande do Sul, Brazil. *Revista Do Instituto de Medicina Tropical de São Paulo*, *44*, 269–272.

Mishra, V., Nag, V. L., Tandon, R., & Awasthi, S. (2010). Response surface methodology-based optimisation of agarose gel electrophoresis for screening and electropherotyping of rotavirus. *Applied Biochemistry and Biotechnology*, *160*, 2322–2331.

Ostrosky-Zeichner, L., Casadevall, A., Galgiani, J. N., Odds, F. C., & Rex, J. H. (2010). An insight into the antifungal pipeline: selected new molecules and beyond. *Nature Reviews Drug Discovery*, *9*(9), 719–727.

Sambrook, J., & Russell, D. W. (2006). Cycle Sequencing: Dideoxy-mediated Sequencing Reactions Using PCR and End-labeled Primers. *Molecular Cloning: A Laboratory Manual, 2*, 12–51.

