Molecular Characterization of Trichophyton Mentagrophyte in Kirkuk City

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Abstract

The current study was conducted to investigate the dermatophytosis fungi on humans in the city of Kirkuk and the prevalence of *Trichophyton mentagrophyte* All samples were examined and diagnosed using traditional methods and using culture media for the fungi, while the isolated *Trichophyton mentagrophyte* were diagnosed using PCR technique for the purpose of supporting laboratory diagnosis The results of the PCR examination were sent to the Genebank website to confirm the type of fungus by comparing it with the fungi recorded on this site and using the Chromas program and Mega-X program. The results of the analysis showed that all isolates were diagnosed to the fungal type *Trichophyton mentagrophytes*.

Keywords : Dermatophytes , Trichophyton mentagrophyte , PCR

Introduction

Dermatophytes are the most common cause of fungal infections worldwide and are a group of filamentous fungi that infect the keratinized outer layer of the skin as well as nails and hair of humans and animals. Dermatophytes include three genera of incomplete fungi, *Trichophyton*, *Microsporum* and *Epidermophyton* On the invasion and growth in the stratum corneum and the high ability to secrete multiple extracellular enzymes, especially keratinase enzyme, which plays an important role in the virulence of these fungi. The infection is generally cutaneous and limited to the non-living stratum corneum due to the inability of the fungi to penetrate the deep tissues or organs of the host. (Al-Muhna. , 2017). Dermatophytes cause fungal infections called dermatophytosis or tinea, whose name depends on the location of the infection in the body, or ring worm, which means ring worms, because the infection often takes the form of a ring with inflammation of the edges and a clear center of normal skin with the presence of fungal elements in an active state on the edge of the lesion, which It is preferable to take the sample from it when microscopic diagnosis. (AL-Janabi, 2014).

Trichophyton mentagrophytes are a common skin fungus that causes skin diseases in humans and other animals (Quiñones *et al.*, 2016). It belongs to the genus *Trichophyton*, of the family Arthrodermataceae, the order Onygenales, of the class Eurotiomycetes, and the phylum

Ascomycota. . *T. mentagrophytes* It is a type of contagious fungi that primarily causes skin diseases such as tinea pedis, tinea corporis, and tinea capitis (Szili & Köhalmi, 2009). It is almost universally prevalent, especially in humid and carbon-rich environments. It is a variable fungus. It has many characteristics that are inconsistent when cultured in a different medium (Kano & Hasegawa, 2014).*T. mentagrophytes* colonies are generally flat in colour, white to cream, with powdery on the granular surface (Hohaus *et al.*, 2003). Reverse pigmentation is usually yellow-brown to reddish-brown Some laboratory cultures show a central fold or develop raised central tufts or polymorphous suede-like areas into smooth areas of the colony (Kano & Hasegawa, 2014) The colony is usually granular in shape It has a powdery appearance due to the numerous unicellular microconidia (spores) formed (Kano & Hasegawa, 2014). Microconidia of *T. mentagrophytes* are usually a smooth-walled glassy formation, spherical to almost spherical in shape, and sometimes pear-shaped (Koch, 2009). While Macroconidia are spindle-shaped, thinor thick-walled with 4-5 cells separated by parallel transverse walls, which grow laterally (Kurtdede *et al.*, 2014).

Materials and methods

Samples Collection

100 clinical samples were collected including (skin scrabs - hair samples - nail clippers) from different age groups and for both sexes from patients referred to the consultant dermatology at Azadi Teaching Hospital Kirkuk, as well as some private clinics for the period from November (2020) to May (2021), where a Clinical examination of those patients by a dermatologist, and a questionnaire was assigned to each patient, which contained some medical and special information about the auditors.

Samples examination

Direct Microscopic examination for samples

Hair, skin and nails samples were examined according to (Koneman *et al.*,1978) and as follows:

1_ A part of the sample (skin, hair, nails) was placed using an inoculation needle on a glass slide and a drop of potassium hydroxide KOH was added at a concentration of 10% Usually, the skin sample is mashed, and 40% concentration of KOH is used for nail samples using a sterile needle, then the cover of the glass slide is placed over the sample.

_2 The prepared glass slide is quietly heated by moving it slightly over the flame of a Bunsen lamp

_3 The sample was left for 21 minutes at room temperature, then the sample was pressed using the base of the needle to brush it on the slide.

_4 The sample was examined under a force microscope (10X) and (40X) to observe the mycelium and other fungal structures.

Culture of Sample

The other part of the samples (skin, hair and nails) were cultured on Sabourad-dextrose agar (SDA) medium containing the antibiotic Chloramphenicol to prevent the growth of bacteria and cyclohexamide to prevent the growth of saprophytic fungi then the plate were incubated at (25) $^{\circ}$ C for two weeks and checked continuously every 2 -3 days .

Molecular characterization

DNA Extraction:

DNA was extracted from colonies of *T. mentagrophyte* using CTE (Chelex®100) supplied by BioRad Company (BioRad, USA) and the extraction was performed according to the company's instructions.

Diagnostic method using the PCR test:

ITS region(internal transcribed spacer) was carried out to confirm the diagnosis of *Trichophyton mentagrophyte* isolates. Fungal DNA was extracted and the ITS target region was amplified using each of the primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G -3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') according to (White *et al.*, 1990).

Preparation of the reaction solution for PCR polymerase chain reactions:

A reaction mixture (PCR 25 microliters) was prepared according to the company's instructions as in the following table:

PCR master mix	Volume
master mix	5 microliters
Forward primer	1 microliters
Reverse primer	1 microliters
Free nucleas water	13 microliters
Genomic DNA	5 microliters
Total	25 microliters

Table (1-1) Volumes of PCR Polymerase Chain Reaction Mixture

Then the components of the PCR reaction mixture mentioned in the above table were put into special 0.2 ml tubes that contain the rest of the PCR reaction components. All tubes were transferred to a vortex exispin centrifuge at a speed of 3000 rpm for three minutes and then

placed in the Thermocycler PCR machine for conducting DNA amplification process according to ideal conditions for thermal cycles.

PCR Thermocycler conditions

The polymerase chain reaction assay was performed using a thermocycler PCR device as shown in the following table:

Table (2-1) Thermal Cycle Conditions for PCR Assay

PCR Step	Repeat cycle	Temperature	Time
Initial denaturation		95 c	3 min
Denaturation	35	95c	30 sec
Annealing		56c	1 min
Extension		72c	50 sec
Final extension		72c	5 min

Analysis of the results of the PCR examination

Electrophoresis was carried out using 1.5% agarose gel electrophoresis, according to the mentioned method, in order to read the result of the PCR product analysis as follows:

1 - Dissolve 1.5 g of agarose gel in 100 ml of buffer TBE solution at a concentration of 1X using a Microwave device for 2 minutes.

2_ The gel was left to cool at 50 °C, then 3 μ l of radioactive DNA dye Ethidium bromide were added and mixed well with the gel.

3_ Pour the agarose gel into the Tray migration tray containing the comb to locate the PCR samples, then leave the gel to solidify at room temperature for 15 minutes, then carefully remove the comb from the gel and transfer to the electrophoresis.

4_ The samples carried the PCR product and were placed in gel pits.

5_ A DNA ladder ranging from 100-1000 bp was used to measure the output of the PCR product and was placed in the first hole.

6_ After the loading process was completed, the agarose gel was immersed in a buffer solution of TBE Buffer at a concentration of 1X and the relay cover was closed, and then the relay was operated using a current of 100 volts and 80 amps for one hour.

7_ After the migration process, the gel containing the PCR product was examined using the V.U light source to determine the product with the measurement unit.

Result and Discussion:

After the isolated fungal species were diagnosed by traditional methods based on determining phenotypic criteria using taxonomic keys as shown previously, and to confirm the validity of their diagnosis using ITS region amplification and using each of the ITS1 primers (5'-TCC GTA GGT GAA CCT GCG G-3'). And ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') according to (White *et al.*, 1990) to diagnose *Trichophyton mentagrophyte*. The PCR technique was used in the current study to diagnose about seven isolates of Fungi based on primers.



Picture(1-1) Electrophoresis on 1.5% agarose gel at voltage 70 for one hour to isolate *Trichophyton mentagrophyte* as, DNA Ladder (550-600 bp).

Diagnosis of the isolates under study based on the sequence of nitrogenous bases of the ITS region:

After the products of DNA replication were sent by PCR technique with the forward and reverse primers Its1 and Its4 to Macrogen Corporation in Korea for the purpose of identifying and sequencing the nitrogenous bases of seven samples of DNA extracted from cultured *Trichophyton* fungi, the sequences were analyzed in BLAST (Basic Local Alignment Search Tool) and by selecting the blastn tool after it was processed using Chromas and Mega-X program, then the results were compared with the data recorded at the National Center for Biotechnology Information (NCBI) of the same isolates registered globally (http://www.ncbi. nlm.nih.gov/).

The results of the sequence analysis of nitrogenous bases with comparison with the isolates registered in NCBI proved that all fungal isolates isolated from infected patients are isolates of the fungus *Trichophyton*, and this result confirms the phenotypic and microscopic diagnosis.

The Nitrogenous Base Recording Document for All *Trichophyton* fungi Isolates at the National Center for Information and Biotechnology (NCBI) clarified the classification of fungi as follows:

Eukaryota; fungi; Dikaria; Ascomycota; Pezizomycotina;Eurotiomycetes; Eurotiomycetidae; Onygenales; Arthrodermataceae; *Trichophyton*

The molecular study also showed accurate diagnosis at the species level by comparing with the isolates registered globally in NCBI, that all seven isolates were diagnosed to the fungal type Trichophyton mentagrophytes. The results obtained are in agreement with the results obtained by (Surendran et al., 2014) and (Maikhan et al., 2018), as in vitro culture and chemical tests identify the types of fungi as well as require more specific methods such as a molecular diagnostic technique for Correct identification of the genera The ITS region of rDNA in dermatophytes has been shown to be useful for identifying and resolving the evolutionary relationship between species of dermatophytes. This result does not agree with (Frías et al., 2020), as among the isolates and diagnosed in vitro, there were two types of isolates belonging to the type Trichophyton, which are Trichophyton interdigitale and Trichophyton mentagrophyte ITS. It is (28S) and the small subunit gene is (18S) and the 5.8S gene is separated by regions called ITS. It is the main site for the identification of fungi in ribosomal genes and because it is present in all organisms and in many copies and is sensitive to polymerase chain reactions and has proven to be particularly useful in elucidating the relationship between Homogeneous species and genera (Fajarningsih., 2016) (Wickes & Wiederhold., 2018). The reason for the presence of mutations in some isolates may be due to resistance to environmental conditions or one of the defensive means of the fungus to resist antibiotics, and this result is consistent with (Mustafa, 2009), who showed that the studied Fungi live in thermal conditions ranging from 25-30 c Which the study was conducted on as well as the differences in the geographical location from which it was isolated or the temperature difference, where the temperature has an effect on the vital activity in the cell directly through its effect on genetic material, enzymes, lipids and cell membrane and its effect on the speed of growth (Norton, 1986).

		ľ	FS internal t	ranscribed spac	er	
No.	Type of	Location	Nucleotide	Sequence ID	Source	Identities
	substitution			with compare		
1	-	-	-	ID:	Trichophyton	100%
				MW898022.1	mentagrophytes	
2	Transvertion	408	C\G	ID:	Trichophyton	99%
				MT106087.1	mentagrophytes	
3	Transition	669	C\T	ID:	Trichophyton	99%
	Transvertion	588	C\A	MK447608.1	mentagrophytes	
	Transition	592	A\G			
	Transition	593	A\G			
	Transvertion	598	C\G			
	Transition	506	G\A			

4	Transvertion	553	G\T	ID: MW682960.1	Trichophyton mentagrophytes	99%
5	Transition	430	G∖A	ID:	Trichophyton	99%
	Transition	392	G∖A	MN737906.1	mentagrophytes	
	Transition	160	G\A			
6	Transition	550	C\T	ID:	Trichophyton	99%
	Transvertion	469	C∖A	OK110588.1	mentagrophytes	
	Transition	387	G∖A			
7	Transvertion	530	T\G	ID:	Trichophyton	99%
	Transition	474	A∖G	OK110592.1	mentagrophytes	
	Transition	387	G∖A			

Table (1-3) of the global isolates and their accession numbers in NCBI, which were compared with them through BLAST website, showing the places of heterogeneity and their location, in addition to the percentage of congruence with the isolates under study.

After completing the data analysis, the isolates under study were registered at the NCBI Information and Biotechnology Center, according to the identification numbers for each isolate, documenting the results obtained in this study as shown in Figure (1-1).

#Accession	Sequence ID Release Date
OK424258	L1 Oct 12, 2021
OK424259	L6 Oct 12, 2021
OK424260	L8 Oct 12, 2021
OK424261	L14 Oct 12, 2021
OK424262	L18 Oct 12, 2021
OK424263	L47 Oct 12, 2021
OK424264	L73 Oct 12, 2021

Figure (1-1) isolates registration numbers under study at the NCBI Information and Biotechnology Center

Molecular analysis of isolates

1-Calculating the ratios of nitrogenous bases (nucleotides).

The ratios of each nitrogen base were calculated for all isolates with the help of the GC Content Calculator Vector Builder program as follows:

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-1-1 Isolation of T. mentagrophytes (OK424258)
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Figure (1-2) indicates the calculation of the ratios of nitrogenous bases for the isolate registered under an identification number in NCBI (OK424258). The length of the DNA segment was 470bp and the ratios of the four bases were as follows: A(21.91% 103) | C(28.72% 135) | G(28.3% 133) | T(21.06% 99).

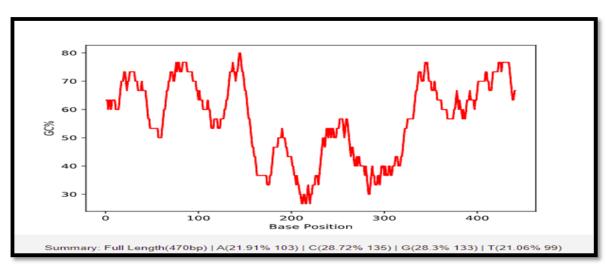


Figure (1-2) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424258).

1-2-Isolation of T. mentagrophytes (OK424259)

Figure (1-3) refers to the calculation of the ratios of nitrogenous bases for the isolate registered under an accession identification number in NCBI (OK424259), as the length of the DNA segment reached 490bp and the ratios of the four bases were as follows: |A(21.02% 103)| C(28.98% 142)|G(28.98% 142)|T(21.02% 103).

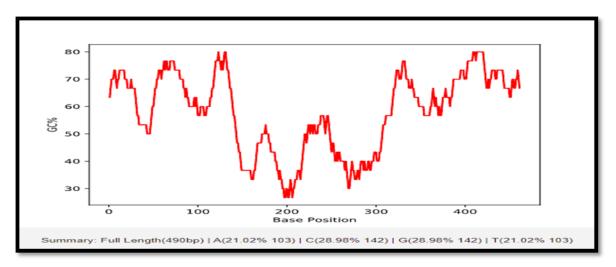


Figure (1-3) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424259)

http://annalsofrscb.ro

-1-3-Isolation of T. mentagrophytes (OK424260)

Figure (1-4) indicates the calculation of the ratios of nitrogenous bases for the isolate registered under an accession identification number in NCBI (OK424260), as the length of the DNA segment reached 424bp and the ratios of the four bases were as follows: |A(19.81% 84)| C(26.89% 114) | G(29.72% 126) | T(23.58% 100).

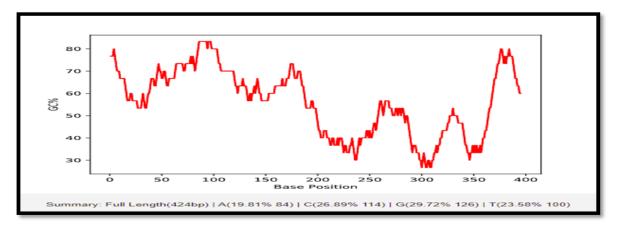


Figure (1-4) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424260)

-1-4-Isolation of T. mentagrophytes (OK424261)

Figure (1-5) refers to the calculation of the ratios of nitrogenous bases for the isolate registered under an accession identification number in NCBI (OK424261), as the length of the DNA segment reached 458bp, and the ratios of the four bases were as follows: |A(22.27% 102)| C(29.26% 134)|G(28.6% 131)|T(19.87% 91).

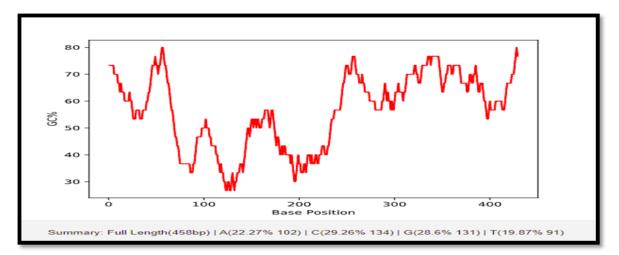


Figure (1-5) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424261)

-1-5-Isolation of T. mentagrophytes (OK424262)

Figure (1-6) indicates the calculation of the ratios of nitrogenous bases for the isolate registered under an accession identification number in NCBI (OK424262), as the length of the DNA segment reached 315bp and the ratios of the four bases were as follows: || A(23.17% 73) | C(26.35% 83) | G(24.44% 77) | T(26.03% 82).

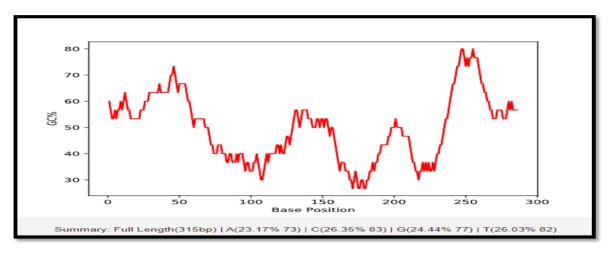


Figure (1-6) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424262)

-1-6-Isolation of T. mentagrophytes (OK424263)

Figure (1-7) indicates the calculation of the ratios of nitrogenous bases for the isolate registered under an accession identification number in NCBI (OK424263), as the length of the DNA segment was 373bp, and the ratios of the four bases were as follows: |A(20.38% 76)|C(26.54% 99)|G(28.95% 108)|T(24.13% 90).

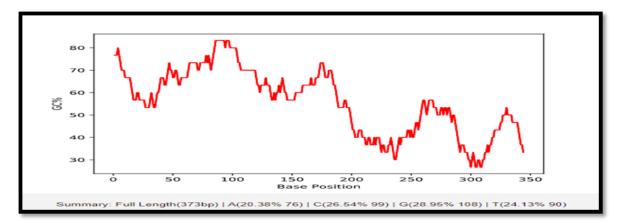


Figure (1-7) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424263)

-1-7-Isolation of T. mentagrophytes (OK424264)

Figure (1-8) refers to the calculation of the ratios of nitrogenous bases for the isolate registered under an accession identification number in NCBI (OK424264), as the length of the DNA segment reached 339bp and the ratios of the four bases were as follows: |A(21.53%73)| C(26.84%91)|G(27.43%93)|T(24.19%82).

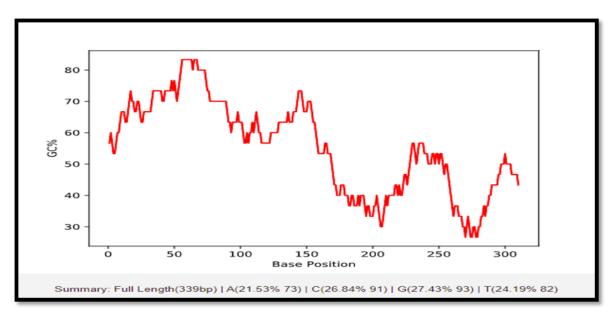


Figure (1-8) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424264)

-1-2- Sequence alignment of nitrogenous bases

-1-2-1- Isolation of T. mentagrophytes (OK424258)

The results of the alignment and comparison of the nitrogenous base sequences of the isolate *T. mentagrophytes* (OK424258) under study showed that they were 100% identical with the isolate T. mentagrophytes registered in France under ID: MW898022.1, as shown by the alignment results shown in Figure (1-9). The absence of any variations (mutations).

Sequer	nce ID:	MW898022.1 L	ength: 626 Number of M	Matches: 1			
Range	1: 41 t	o 510 GenBank	<u>Sraphics</u>		V Next	Match	A Previous Match
Score 869 bit	ts(470)	Expect 0.0	Identities 470/470(100%)	Gaps 0/470(0%)	Strand Plus/P	lus	
Query Sbjct	1 41		AACGTCCGTCAGGGGTGAGCA			60 100	
Query Sbjct	61 101		ACTCGGTTGCCTCGGCGGGCC			120 160	
Query Sbjct	121 161	CGAGCCTCTCTTTA	GTGGCTAAACGCTGGACCGCG	CCCGCCGGAGGACAGACG	Caaaaaa	180 220	
Query Sbjct	181 221		SCTGTCAGTCTGAGCGTTAGC			240 280	
Query Sbjct	241 281		GGTTCCGGCATCGATGAAGAA			300 340	
Query Sbjct	301 341		CGTGAATCATCGAATCTTTGA			360 400	
Query Sbjct	361 401		STTCGAGCGTCATTTCAGCCC			420 460	
Query	421 461		CCCGTTTTTGGGGGGTGCGGGA		70 10		

Figure (1-9) Alignment of nitrogenous base sequences in BLAST between the studied isolate *T. mentagrophytes* (OK424258) and the higher isolate identical to *T. mentagrophytes* registered in France under an accession ID: MW898022.1.

-1-2-2- Isolation of T. mentagrophytes (OK424259)

The results of the alignment and comparison of the nitrogenous base sequences of the isolate *T. mentagrophytes* (OK424259) under study showed that it was 99% identical with the isolate *T. mentagrophytes* registered in Poland under ID: MT106087.1, as indicated by the alignment results shown in figure (1-10).) The presence of one heterogeneity (one mutation).

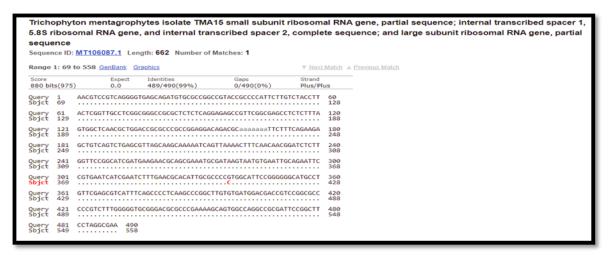


Figure (1-10) Alignment of the nitrogenous base sequences in BLAST between the studied isolate *T. mentagrophytes* (OK424259) and the higher isolate identical to *T. mentagrophytes* registered in Poland under an accession ID: MT106087.1.

-1-2-3- Isolation of T. mentagrophytes (OK424260)

The results of the alignment and comparison of the nitrogenous base sequences of the isolate *T. mentagrophytes* (OK424260) under study showed that they were 99% identical with the isolate *T. mentagrophytes* registered in Germany under ID: MK447608.1 as indicated by the alignment results shown in figure (1-11). The presence of 6 mutations.

Sequer	-	al sequence MK447608.1 Le	ngth: 954 Number o	f Matches: 1			
Range	1: 275	to 698 GenBank	Graphics		Vext	Match	Previous Match
Score 750 bit	s(406)	Expect	Identities 418/424(99%)	Gaps 0/424(0%)	Strand Plus/Minu	IS	
Query Sbjct	57 698					116 639	
Query Sbjct	117 638		CACTGCTTTTCGGGCGCG			176 579	
Query Sbjct	177 578		ACACAAGCCGGGCTTGA			236 519	
Query S <mark>bjct</mark>	237 518		AGGGGGCGCAATGTGCG			296 459	
Query Sbjct	297 458		TTATCGCATTTCGCTGC			356 399	
Query Sbjct	357 398		TTTAACTGATTTTTGCT			416 339	
Query Sbjct	417 338		GCGTCTGTCCTCCGGCG			476 279	
Query Sbjct	477 278	AGAG 480					

Figure (1-11) Alignment of nitrogenous base sequences in BLAST between the studied isolate *T. mentagrophytes* (OK424260) and the higher isolate identical to *T. mentagrophytes* registered in Germany under ID: MK447608.1.

-1-2-4- Isolation of T. mentagrophytes (OK424261)

The results of the alignment and comparison of the nitrogenous base sequences of the isolate *T. mentagrophytes* (OK424261) under study showed that it was 99% identical with the isolate *T. mentagrophytes* registered in India under ID: MW682960.1 as shown by the alignment results shown in figure (1-12) The presence of a single mutation.

Sequer	ice ID:	MW682960.1 Le	ngth: 595 Number of M	latches: 1			
Range	1: 113	to 570 GenBank	Graphics		V Next	Match 🔺	Previous Match
Score 841 bit	ts(455)	Expect 0.0	Identities 457/458(99%)	Gaps 0/458(0%)	Strand Plus/Pl	us	
Query	1 113		CCAGGAGAGCCGTTCGGCGA			60 172	
Query	61 173		AGGACAGACGCaaaaaaaTT			120 232	
Query	121 233		ATCAGTTAAAACTTTCAACA			180 292	
Query Sbjct	181 293		AATGCGATAAGTAATGTGAA			240 352	
Query Sbjct	241 353		TGCGCCCCTGGCATTCCGG			300 412	
Query Sbjct	301 413		CGGCTTGTGTGATGGACGAC			360 472	
Query	361 473		AAAAGCAGTGGCCAGGCCGC			420 532	
Query Sbjct	421 533		TCCATGACCGGCCGCCCTGG				

Figure (1-12) Sequence alignment of nitrogenous bases in BLAST between the studied isolate *T. mentagrophytes* (OK424261) and the higher matching isolate *T. mentagrophytes* registered in India under an accession ID: MW682960.1.

-1-2-5- Isolation of *T. mentagrophytes* (OK424262)

The results of the alignment and comparison of the nitrogenous base sequences of the isolate *T*. *mentagrophytes* (OK424262) under study showed that it was 99% identical with the isolate *T*. *mentagrophytes* registered in China under ID: MN737906.1 as shown by the alignment results shown in figure (1-13). The presence of 3 variants (mutations).

internal	tra	nscribed space	nytes isolate BMU er 2, complete see ngth: 645 Number of	quence; and large		
Range 1:	141	to 454 GenBank	<u>Graphics</u>		Vext Match	Previous Match
Score 558 bits(3	302)	Expect 7e-159	Identities 311/315(99%)	Gaps 1/315(0%)	Strand Plus/Minus	
			GGCTTGAGGAGCTGAAATO			
			ATGTGCGTTCAAAGATT			
	25 35		TTCGCTGCGTTCTTCATCO			
	85 75		TTTTTGCTTGCTAACGCT			
Query 4 Sbjct 2			TCCGGCGGGCGCGGTCC/			
		CCGAACGGCTCTCCT	519 141			

Figure (1-13) Alignment of the nitrogenous base sequences in BLAST software between the studied isolate *T. mentagrophytes* (OK424262) and the higher matching isolate *T. mentagrophytes* registered in China under an accession ID: MN737906.1.

-1-2-6- Isolation of T. mentagrophytes (OK424263)

The results of the alignment and comparison of the nitrogenous base sequences of the isolate *T. mentagrophytes* (OK424263) under study showed that they were 99% identical with the isolate *T. mentagrophytes* recorded in Iran under ID: OK110588.1 as shown by the alignment results shown in figure (1-14). The presence of 3 variants (mutations).

transc	ribed	spacer 2, co	ohytes isolate M16 mplete sequence; ength: 648 Number of	and large subu		
Range 1	: 207 (o 579 GenBank	Graphics		Vext Match	Previous Match
Score 673 bits	s(364)	Expect 0.0	Identities 370/373(99%)	Gaps 0/373(0%)	Strand Plus/Minus	
Query Sbjct	1 579		GCCCGTCCTGGAGGCG			60 520
Query Sbjot	61 519		GGCCACTGCTTTTCGGG			120 460
Query Sbjct	121 459		atcacacaagccgggct			180 400
Query Sbjet	181 399		ACCAGGGGGCGCAATGTC			240 340
Query Sbjct	241 339		TACTTATCGCATTTCGC			300 280
Query Sbjct	301 279	TCCGTTGTTGAA	AGTTTTAACTGATTTTT			360 220
Query Sbjct	361 219	GAAAGAATTTTT	T 373 . 207			

Figure (1-14) Alignment of the nitrogenous base sequence in BLAST between the studied isolate *T. mentagrophytes* (OK424263) and the higher isolate matching *T. mentagrophytes* registered in Iran under an accession ID: OK110588.1.

-1-2-7- Isolation of T. mentagrophytes (OK424264)

The results of the alignment and comparison of the nitrogenous base sequences of the isolate *T. mentagrophytes* (OK424264) under study showed that it was 99% identical with the isolate *T. mentagrophytes* recorded in Iran under ID: OK110592.1 as shown by the alignment results shown in figure (1-15). The presence of 3 variants (mutations).

		5 1				partial sequence; 5.8S ribosomal RNA gene and intern
			igth: 648 Number of M			
Range 1	: 211 t	to 549 GenBank	Graphics		Vext Match	Previous Match
Score 599 bits	s(663)	Expect 1e-170	Identities 336/339(99%)	Gaps 0/339(0%)	Strand Plus/Minus	_
Query	1 549		ATTCGCCGAGGAAGCCGG			60 490
uery bjct	61 489		CCGAAGACGGGGGGGCGCCC			120 430
uery bjet	121 429		GACGCTCGAACAGGCATC			180 370
uery bjct	181 369		GATGATTCACGGAATTCI			240 310
uery bjct	241 309		ATGCCGGAACCAAGAGAI			300 250
bjct	301 249		AGACTGACAGCTCTTCT			

Figure (1-15) Alignment of the sequence of nitrogenous bases in BLAST program between the isolate under study *T. mentagrophytes* (OK424264) and the higher isolate identical to *T. mentagrophytes* registered in Iran under an accession ID: OK110592.1.

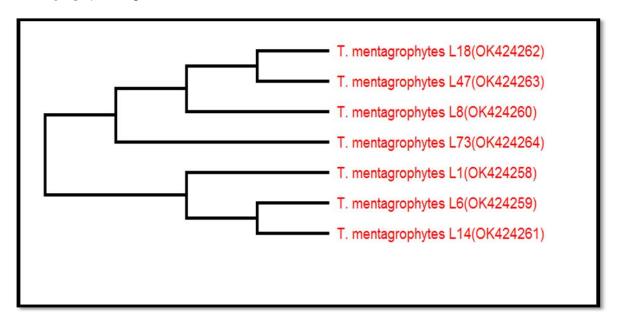


Figure (1-16) Phylogenetic tree showing the evolutionary relationship between the types of *T*. *mentagrophytes* under study using Mega-x program and by the neighbor-joining method.

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Figure (1-17) Alignment of the sequence of nitrogenous bases between the isolates under study using the Mega -x . program.

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