Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

Molecular Characterization of *Trichophyton Mentagrophyte* in Kirkuk City

Huda A. Abdulkareem[1] , Hiba Y. Khalaf[2] , Bari L. Mohammed[3]

[1] Student , [2]Assist. Prof.,[3] Assist. Prof. Dr. , [2]Department of Microbiology, College of Veterinary Medicine, University of Tikrit **,**[3]Department of biology,college of science,university of kirkuk

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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

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, thin- or 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.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

_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) °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:

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

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**

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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

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 µl 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.

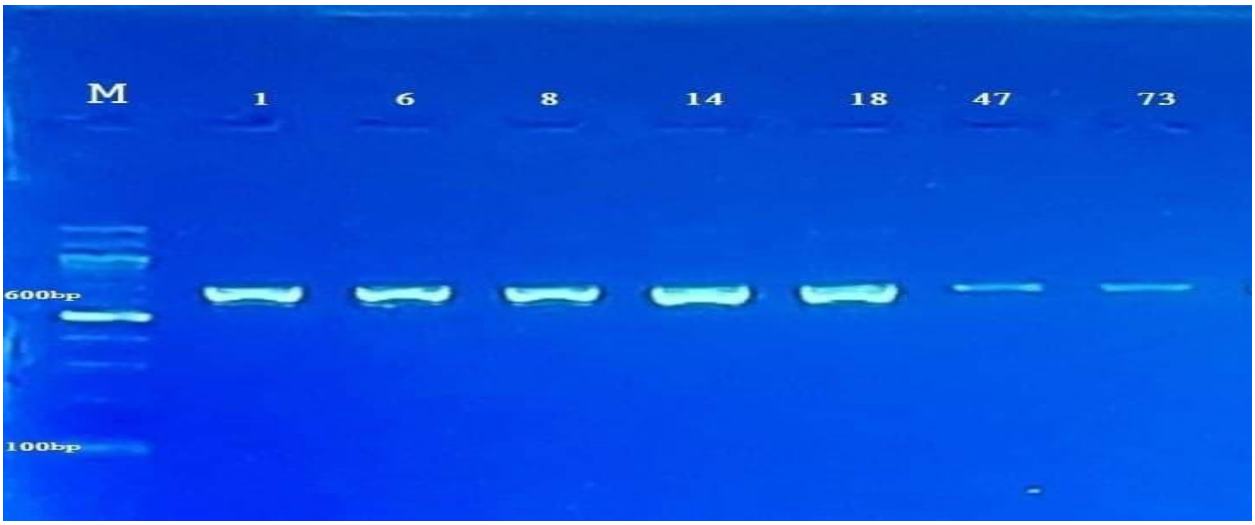
6_ After the loading process was completed, the agarose gel was immersed in a buffer solution of TBE Buffer at a concentration of 1X and the relay cover was closed, and then the relay was operated using a current of 100 volts and 80 amps for one hour.

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.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

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.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

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).

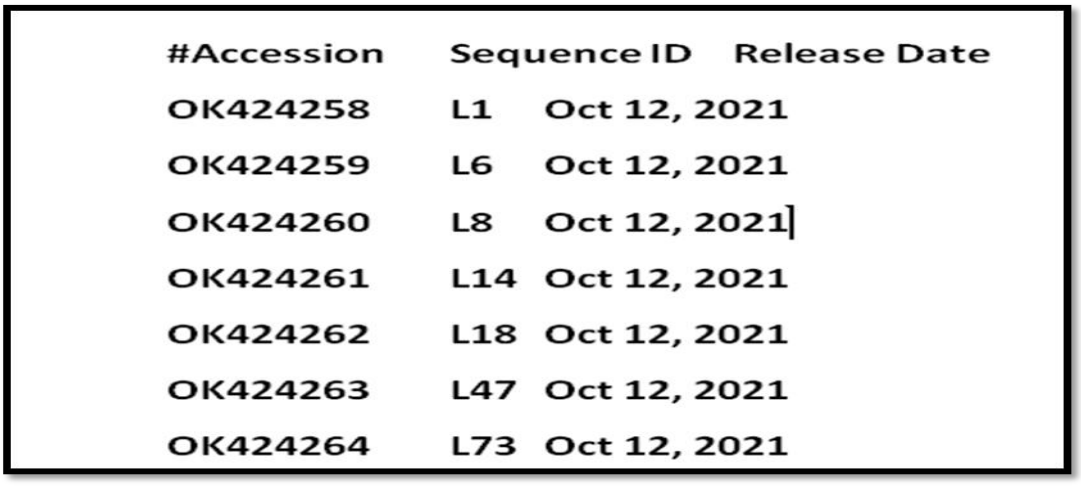
ITS internal transcribed spacer						
No.	**Type of substitution**	**Location**	**Nucleotide**	**Sequence ID with compare**	**Source**	**Identities**
1	**-**	**-**	**-**	**ID: MW898022.1**	***Trichophyton mentagrophytes***	**100%**
2	**Transvertion**	**408**	**C\G**	**ID: MT106087.1**	***Trichophyton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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

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

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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

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).

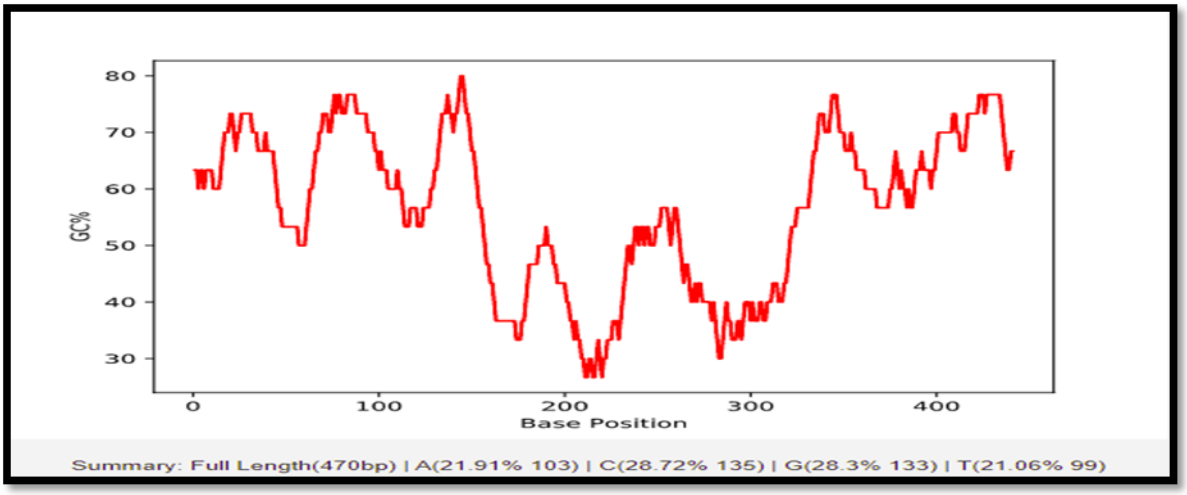


Figure (1-2) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424258).

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).

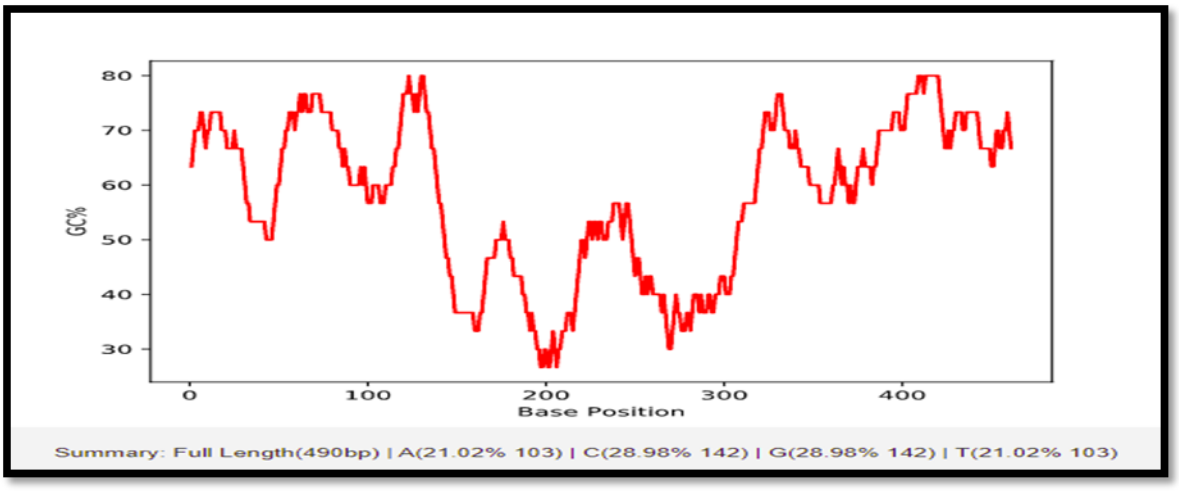


Figure (1-3) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424259)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

-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).

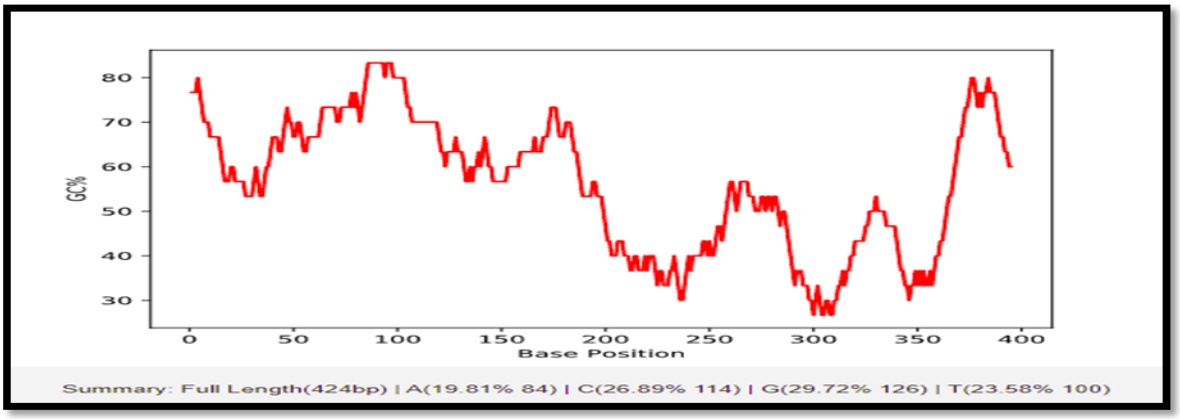


Figure (1-4) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424260)

-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).

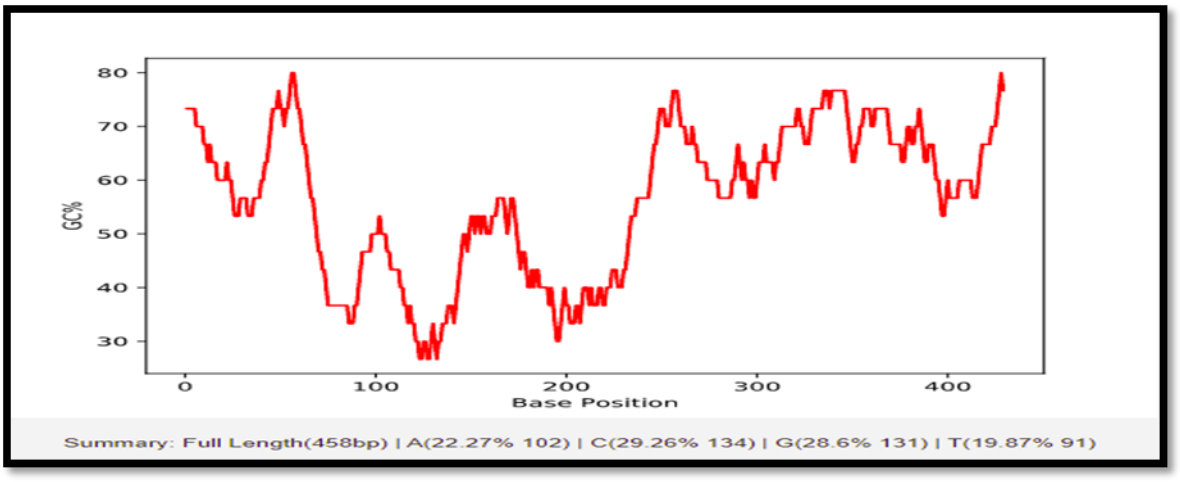


Figure (1-5) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424261)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

-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).

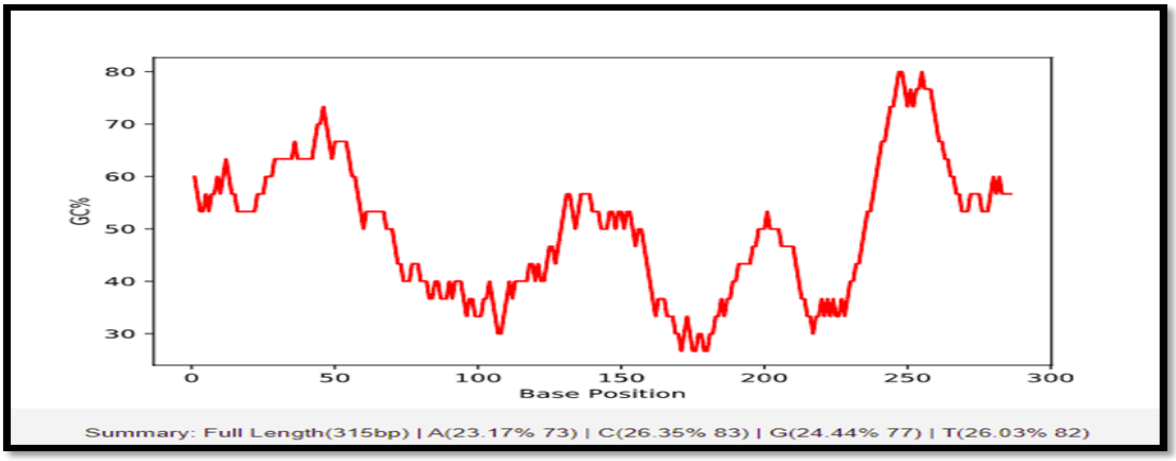


Figure (1-6) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424262)

-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).

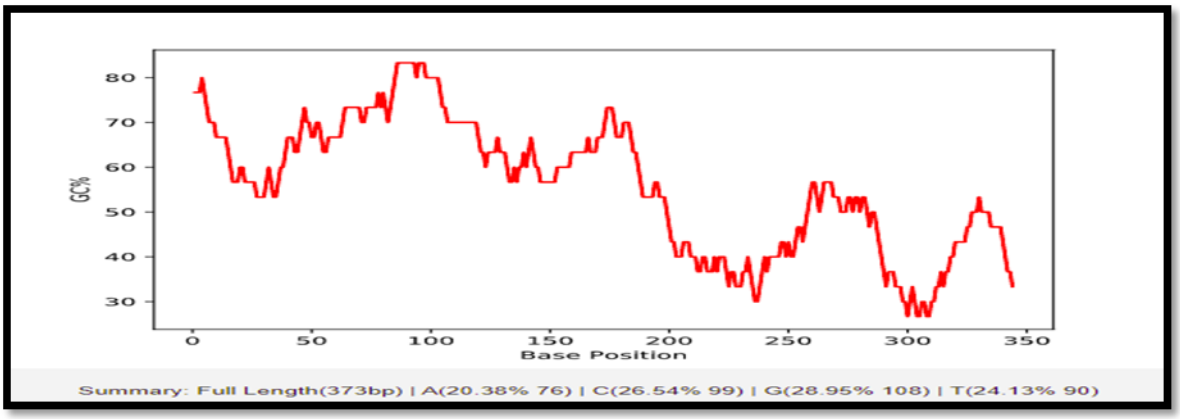


Figure (1-7) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424263)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

-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).

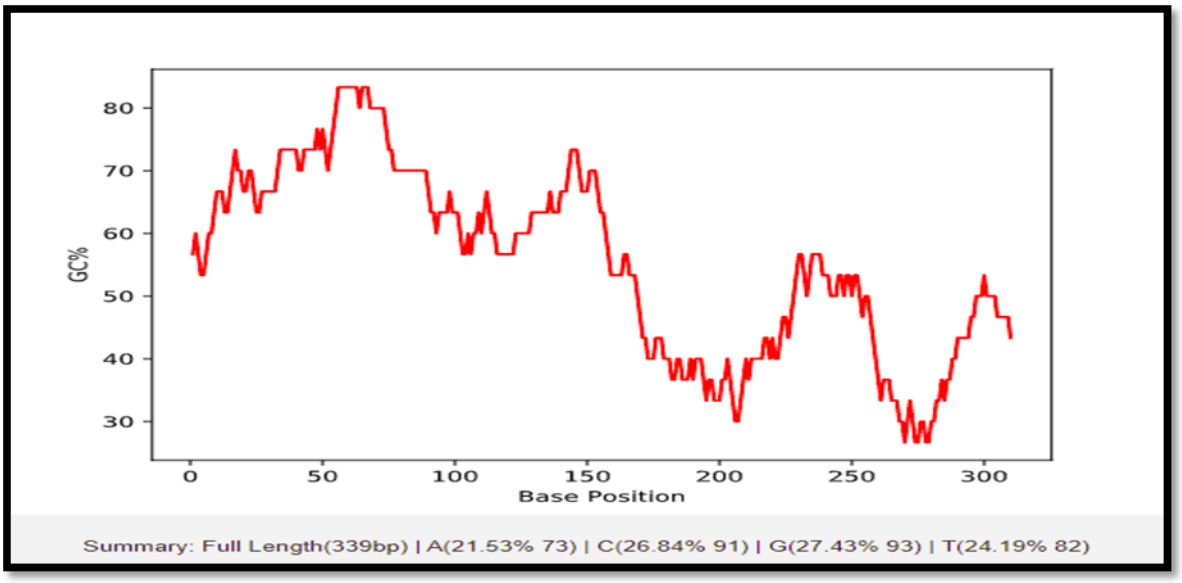


Figure (1-8) Calculation of the ratios of nitrogenous bases for isolate *T. mentagrophytes* (OK424264)

-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).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

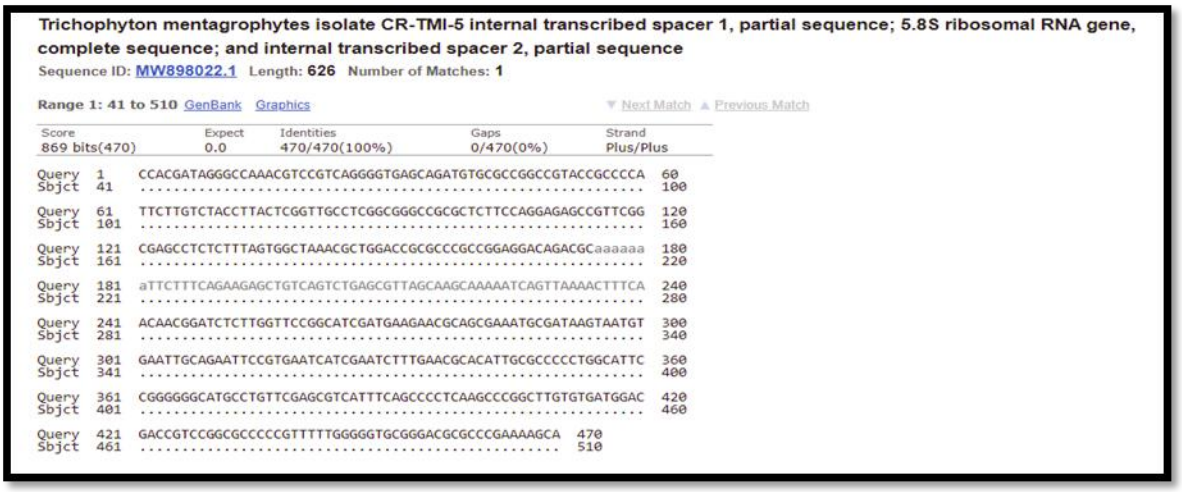


Figure (1-9) Alignment of nitrogenous base sequences in BLAST between the studied isolate *T. mentagrophytes* (OK424258) and the higher isolate identical to *T. mentagrophytes* registered in France under an accession ID: MW898022.1.

-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).

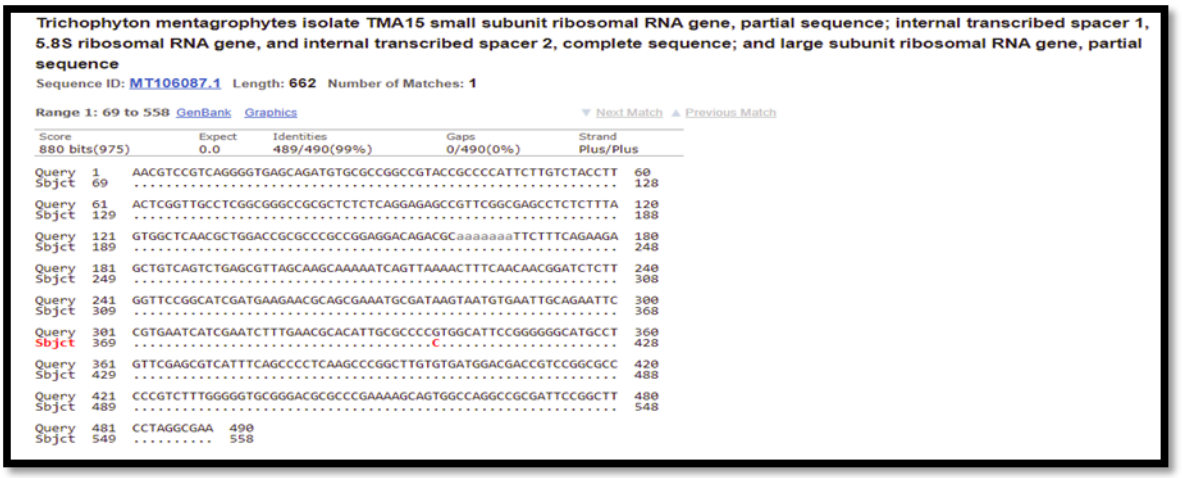


Figure (1-10) Alignment of the nitrogenous base sequences in BLAST between the studied isolate *T. mentagrophytes* (OK424259) and the higher isolate identical to *T. mentagrophytes* registered in Poland under an accession ID: MT106087.1.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

-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.

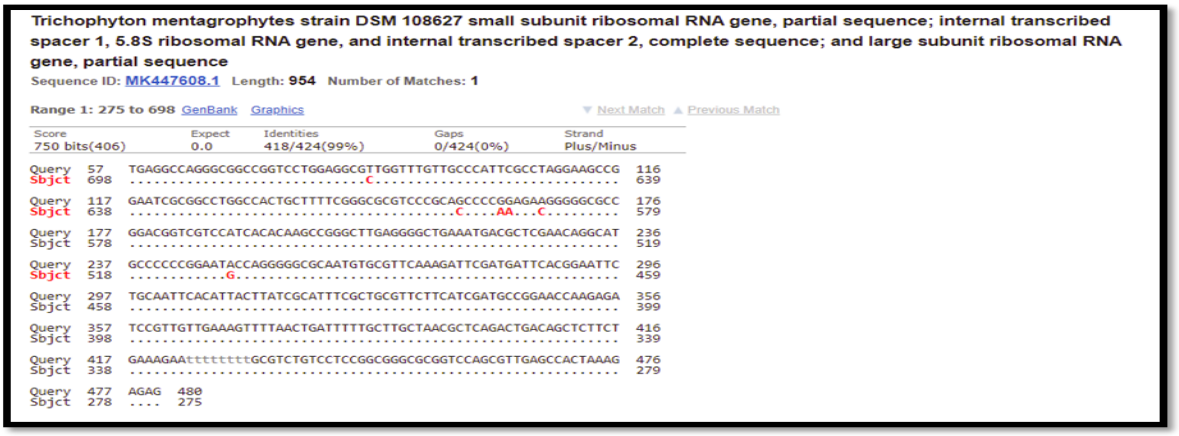


Figure (1-11) Alignment of nitrogenous base sequences in BLAST between the studied isolate *T. mentagrophytes* (OK424260) and the higher isolate identical to *T. mentagrophytes* registered in Germany under ID: MK447608.1.

-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.

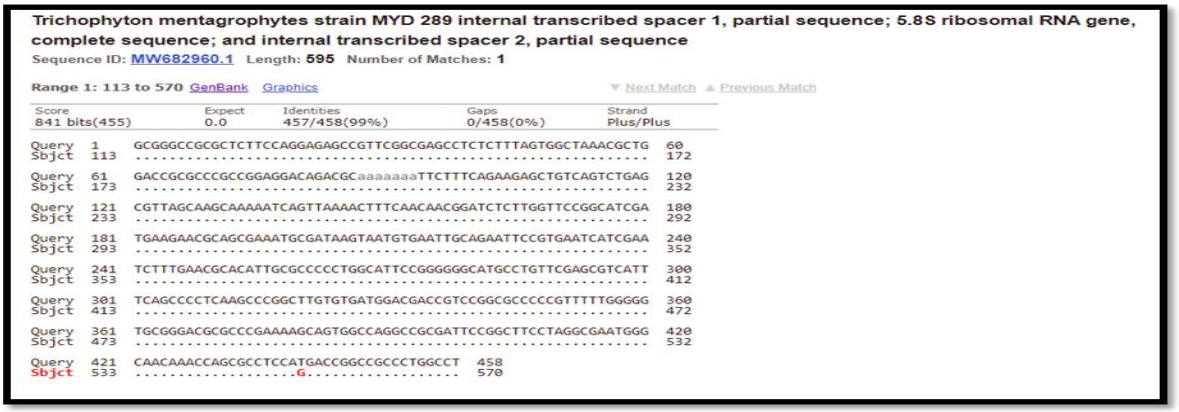


Figure (1-12) Sequence alignment of nitrogenous bases in BLAST between the studied isolate *T. mentagrophytes* (OK424261) and the higher matching isolate *T. mentagrophytes* registered in India under an accession ID: MW682960.1.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

-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).

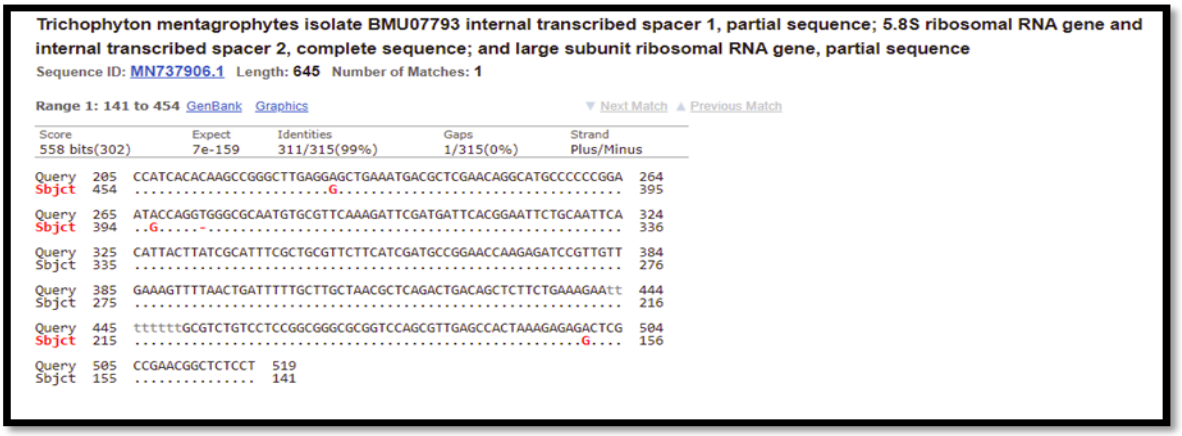


Figure (1-13) Alignment of the nitrogenous base sequences in BLAST software between the studied isolate *T. mentagrophytes* (OK424262) and the higher matching isolate *T. mentagrophytes* registered in China under an accession ID: MN737906.1.

-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).

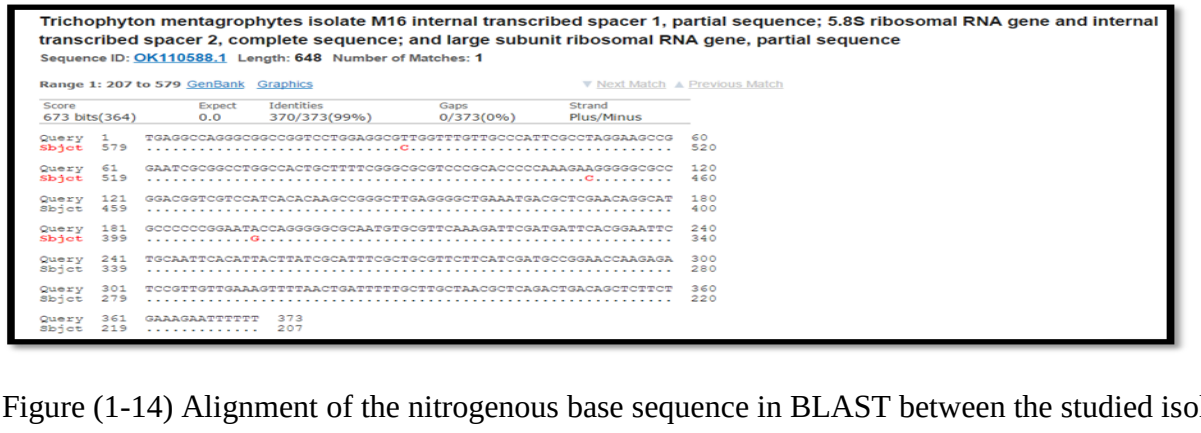


Figure (1-14) Alignment of the nitrogenous base sequence in BLAST between the studied isolate *T. mentagrophytes* (OK424263) and the higher isolate matching *T. mentagrophytes* registered in Iran under an accession ID: OK110588.1.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

-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).

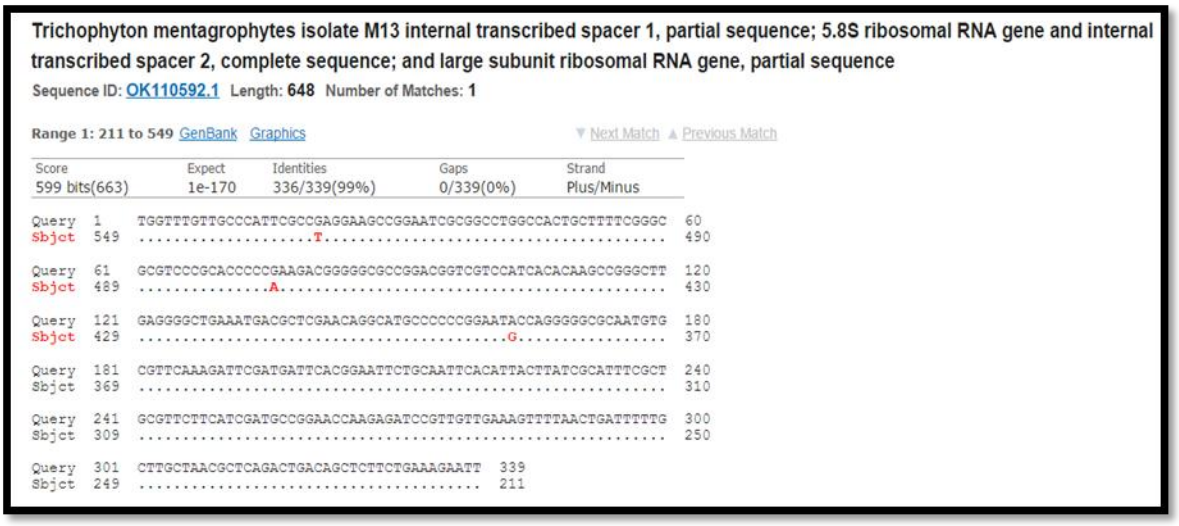


Figure (1-15) Alignment of the sequence of nitrogenous bases in BLAST program between the isolate under study *T. mentagrophytes* (OK424264) and the higher isolate identical to *T. mentagrophytes* registered in Iran under an accession ID: OK110592.1.

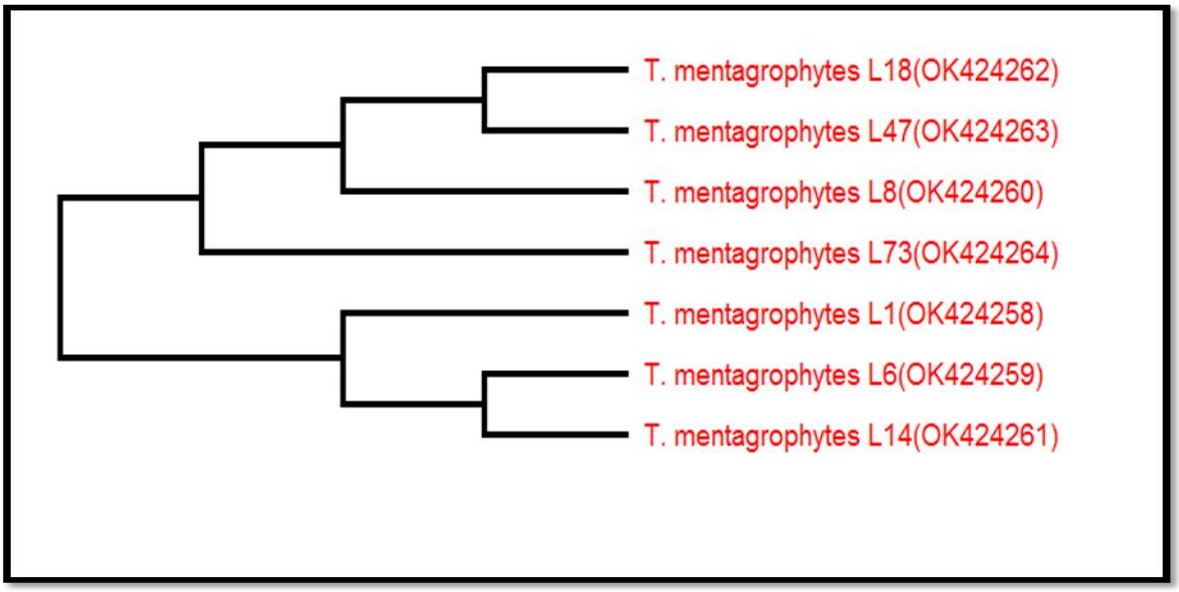


Figure (1-16) Phylogenetic tree showing the evolutionary relationship between the types of *T. mentagrophytes* under study using Mega-x program and by the neighbor-joining method.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

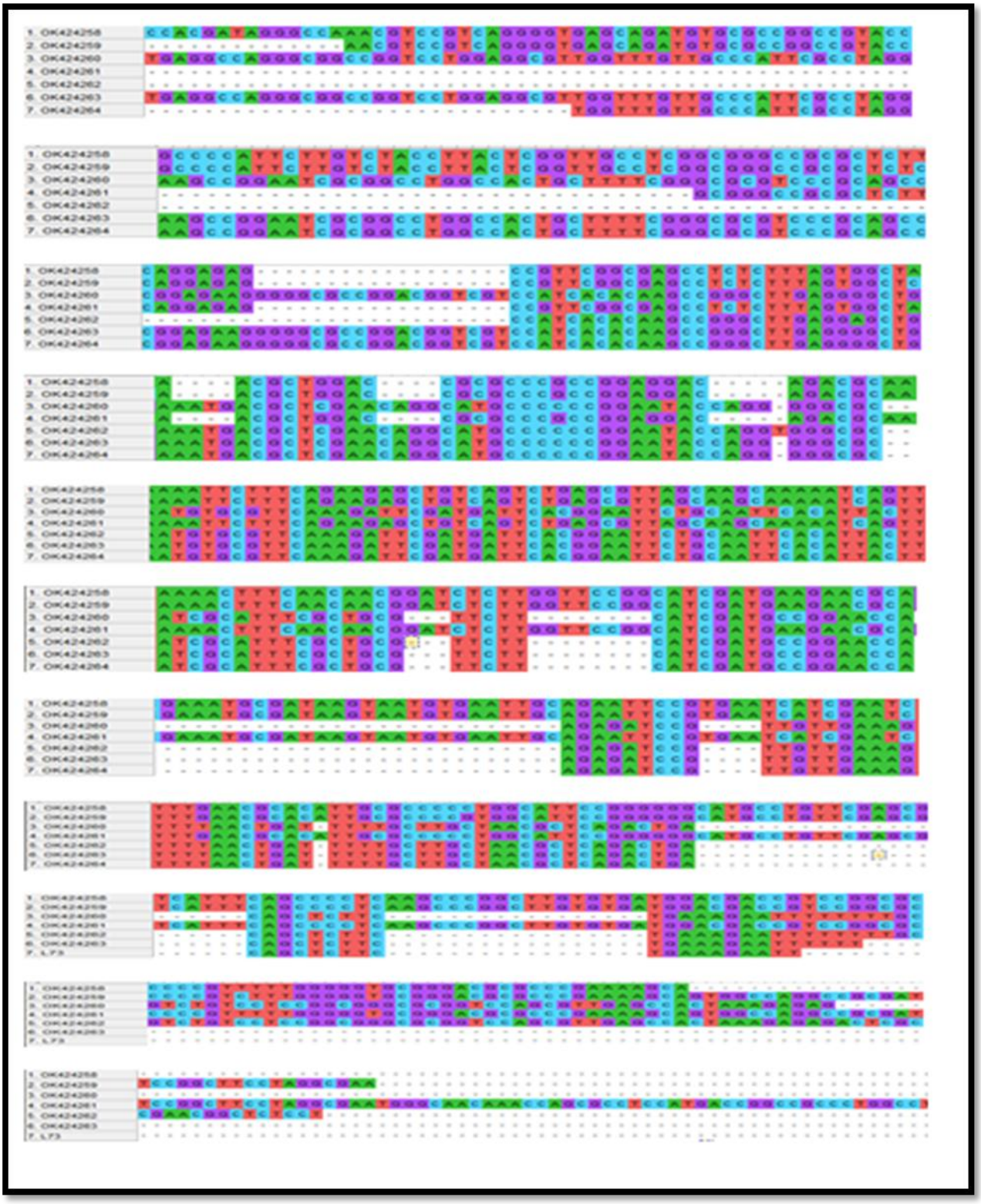


Figure (1-17) Alignment of the sequence of nitrogenous bases between the isolates under study using the Mega -x . program.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

REFERANCE:

1-AL-Muhna , B. M. M. .(2017). Myco-biosynthesis of silver nanoparticles by Aspergillus niger and evaluation of theirs antifungal activity in Dermatophytes spp. and Candida albicans. Doctoral thesis, College of Medicine , University of Al-Qadisiyah .

2-_Al-Janabi, A. H. (2014). Dermatophytosis: causes, clinical features, signs and treatment. *J Symptoms Signs*, *3*(3), 200-3.

3- Quiñones, C., Hasbún, P., & Gubelin, W. (2016). Tinea incognito due to Trichophyton mentagrophytes: case report. Medwave, 16(10), e6598-e6598.

4- Szili, M., & Köhalmi, I. (2009). Endemic Trichophyton Mentagrophytes Infection of Rabbit Origin: Endemische Trichophyton mentagrophytes-Infektion, verursacht durch Kaninchen. Mycoses, 24(7), 412-420.

5- Kano, R., & Hasegawa, A. (2014). Historic Topics on Classification of Trichophyton mentagrophytes Complex. Medical Mycology Journal, 55(2), J73-J77.

6- Hohaus, K., Vennewald, I., & Wollina, U. (2003). Deep mycosis caused by Trichophyton mentagrophytes in a diabetic patient. Fallbericht. Tiefe Mykose durch Trichophyton mentagrophytes bei einem Diabetiker. Mycoses, 46(8), 337-339.

7- Koch, H. (2009). Bestimmung von Trichophyton rubrum und Trichophyton mentagrophytes. Mycoses, 15(8), 337-338.

8- Kurtdede, A., Haydardedeoglu, A., Alihosseini, H., & Colakoglu, E. (2014). Dermatophytosis caused by Trichophyton mentagrophytes var. erinacei in a dog: a case report. Veterinární Medicína, 59(No. 7), 349-351.

9- White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications, 18(1), 315-322.

10- Maikhan, H. K., Mohammad-Amin, S. M., & Tawfeeq, H. M. (2018). Morphological and Molecular Identification of Trichophyton mentagrophytes Isolated from Dermatophytes Patients in Garmian Area. *Zanco Journal of Pure and Applied Sciences*, *30*(4), 1-10.

11- Frías-De-León, M. G., Martínez-Herrera, E., Atoche-Diéguez, C. E., González-Cespón, J. L., Uribe, B., Arenas, R., & Rodríguez-Cerdeira, C. (2020). Molecular identification of isolates of the Trichophyton mentagrophytes complex. *International journal of medical sciences*, *17*(1), 45.

12- Wickes, B. L., & Wiederhold, N. P. (2018). Molecular diagnostics in medical mycology. *Nature communications*, *9*(1), 1-13.

13- Fajarningsih, N. D. (2016). Internal Transcribed Spacer (ITS) as DNA barcoding to identify fungal species: a review. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, *11*(2), 37-44.

14- Surendran , K.A.K., BHAT, R.M., BOLOOR, R., NANDAKISHORE, B. AND SUKUMAR, D .(2014). A clinical and mycological study of dermatophytic infections. Indian journal of dermatology, 59(3),.262.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1933 - 1950
Received 05 July 2021; Accepted 01 October 2021.

15- Koneman, E.W.; Roberts, G.D. and Wright, S.H.(1978). Practical Laboratory mycology. The Williams and Wilkins company Baltmore, USA Journal of Medicinal Plants Research.

16- Norton,C.F. (1986). How to grow and study microorganisms.In: microbiology.2nd ed.,Addison – Wesley Publishing ,USA,PP.165-193.

17- Mustafa, B. L. M. (2009). Epidemiology and diagnostic study of dermatophytes in Kirkuk city and effect onion plant extracts on isolated fungi. Master's thesis, College of Science, Tikrit University, Republic of Iraq.